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# The Bioenvironmental Impact of a Coal-Fired Power Plant, Colstrip, Montana, December 1977

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The EPA has recongnized the need for a rational approach to the incorporation of ecological impact information into power facility siting decisions in the northern great plains. Research funded by the Colstrip, Coal-fired Power Plant Project is a first attempt to generate methods to predict the bioenvironmental effects of air pollution before damage is sustained. Pre-construction documentation of the environmental characteristics of the grassland ecosystem in the vicinity of Colstrip, Montana began in the summer of 1974. Since then, key characteristics of the ecosystem have been monitored regularly to detect possible pollution impacts upon plant and animal community structure.

In the summer of 1975, field stressing experiments were begun to provide the data necessary to develop dose-response models for $SO_2$ stress on a grassland ecosystem. These experiments involve continuous stressing of one acre grassland plots with measured doses of $SO_2$ during the growing season (usually April through October).

Results of the 1975 field season's investigations are summarized in this publication. The six-year project will terminate in 1980 and a final report will be published after data analyses are complete.

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# THE BIOENVIRONMENTAL IMPACT OF A COAL-FIRED POWER PLANT

## Third Interim Report, Colstrip, Montana

December 1977

Edited by
Eric M. Preston and Robert A. Lewis
Ecological Effects Research Division
Corvallis Environmental Research Laboratory
Corvallis, OR 97330

CORVALLIS ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CORVALLIS, OR 97330

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# FOREWORD

Effective regulatory and enforcement actions by the Environmental Protection Agency would be virtually impossible without sound scientific data on pollutants and their impact on environmental stability and human health. Responsibility for building this data base has been assigned to EPA's Office of Research and Development and its 15 major field installations, one of which is the Corvallis Environmental Research Laboratory (CERL).

The primary mission of the Corvallis Laboratory is research on the effects of environmental pollutants on terrestrial, freshwater, and marine ecosystems; the behavior, effects and control of pollutants in lake systems; and the development of predictive models on the movement of pollutants in the biosphere.

The Colstrip, Coal-fired Power Plant Project is the first attempt to generate methods to predict the bioenvironmental effects of air pollution before damage is sustained. The methods are to be framed within an overall facility siting strategy for coal-fired power plants in the northern plains. This will permit planners to assess the ecological impact of energy conversion activities on grasslands prior to site selection. If environmental impacts can be predicted, mitigation efforts can be designed to minimize the adverse effects of power plant construction and operation.

A. F. Bartsch
Director, CERL

# ABSTRACT

The Environmental Protection Agency (EPA) has recognized the need for a rational approach to the incorporation of ecological impact information into power facility siting decisions in the northern great plains. The Colstrip Coal-fired Power Plant Project (CFPPP) is one aspect of the agency's response to this need. Research funded by the CFPPP is a first attempt to generate methods to predict the bioenvironmental effects of air pollution before damage is sustained. The methods are to be framed within an overall facility siting strategy for coal-fired power plants in the northern plains. This document describes the progress of the investigation.

Pre-construction documentation of the environmental characteristics of the grassland ecosystem in the vicinity of Colstrip, Montana began in the summer of 1974. This documentation continued until Colstrip generating unit 1 began operation in September, 1975. Since then, key characteristics of the ecosystem have been monitored regularly to detect possible pollution impacts upon plant and animal community structure; primary production, invertebrate animal consumers, and decomposers; plant and animal diseases; both beneficial and harmful insects; indicators and predictors of pollution (e.g., lichens and honeybees); physiological responses of plants and vertebrate animals; insect behavior (mainly of honeybees) and production; the behavior, reproduction and development, population biology, health and condition of vertebrate animals.

In the summer of 1975, field stressing experiments were begun to provide the data necessary to develop dose-response models for $SO_2$ stress on a grassland ecosystem. These experiments involve continuous stressing of one acre grassland plots with measured doses of $SO_2$ during the growing season (usually April through October). Results of the 1975 field season investigations are summarized in this publication. The six-year project will terminate in 1980 and a final report will be published after data analyses are complete.

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# LIST OF CONTRIBUTORS

N. L. Abshire
U.S. Dept. of Commerce
N.O.A.A.
Environmental Research Laboratories
Boulder, Colorado 80302

L. Bard
U.S. EPA/CERL
200 S.W. 35th St.
Corvallis, Oregon 97330

J. J. Bromenshenk
Environmental Studies Laboratory
University of Montana
Missoula, Montana 59801

J. D. Chilgren
U.S. EPA/CERL
200 S.W. 35th St.
Corvallis, Oregon 97330

M. Coughenour
Natural Resources Ecology Laboratory
Colorado State University
Fort Collins, Colorado 80521

V. E. Derr
U.S. Dept. of Commerce
N.O.A.A.
Environmental Research Laboratories
Boulder, Colorado 80302

J. L. Dodd
Natural Resources Ecology Laboratory
Colorado State University
Fort Collins, Colorado 80521

S. Eversman
Dept. of Biology
Montana State University
Bozeman, Montana 59715

R. W. Field
U.S. EPA/CERL
200 S.W. 35th St.
Corvallis, Oregon 97330

N. R. Glass
U.S. EPA/CERL
200 S.W. 35th St.
Corvallis, Oregon 97330

C. C. Gordon
Environmental Studies Laboratory
University of Montana
Missoula, Montana 59801

R. K. Heitschmidt
Natural Resources Ecology Laboratory
Colorado State University
Fort Collins, Colorado 80521

M. D. Kern
Dept. of Biology
The College of Wooster
Wooster, Ohio 44691

W. K. Lauenroth
Natural Resources Ecology Laboratory
Colorado State University
Fort Collins, Colorado 80521

J. J. Lee
U.S. EPA/CERL
200 S.W. 25th St.
Corvallis, Oregon 97330

J. W. Leetham
Natural Resources Ecology Laboratory
Colorado State University
Fort Collins, Colorado 80521

W. C. Leininger
Dept. of Animal and Range Sciences
Montana State University
Bozeman, Montana 59715

R. A. Lewis
U.S. EPA/CERL
200 S.W. 35th St.
Corvallis, Oregon 97330

G. T. McNice
U.S. Dept. of Commerce
N.O.A.A.
Environmental Research Laboratories
Boulder, Colorado 80302

M. L. Morton
Dept. of Biology
Occidental State College
1600 Campus Rd.
Los Angeles, California 90041

E. M. Preston
U.S. EPA/CERL
200 S.W. 35th St.
Corvallis, Oregon 97330

R. Pueschel
U.S. Dept of Commerce
N.O.A.A.
Environmental Research Laboratories
Boulder, Colorado 80302

P. M. Rice
Environmental Studies Laboratory
University of Montana
Missoula, Montana 59801

J. E. Taylor
Dept. of Animal and Range Sciences
Montana State University
Bozeman, Montana 59715

D. T. Tingey
U.S. EPA/CERL
200 S.W. 35th St.
Corvallis, Oregon 97330

P. C. Tourangeau
Environmental Studies Laboratory
University of Montana
Missoula, Montana 59801

C. Van Valin
U.S. Dept. of Commerce
N.O.A.A.
Environmental Research Laboratories
Boulder, Colorado 80302

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# INTRODUCTION

# ASSESSMENT OF ECOLOGICAL IMPACT FROM THE OPERATION OF A COAL-FIRED POWER PLANT IN THE NORTHCENTRAL GREAT PLAINS

by

R. A. Lewis, E. M. Preston, and N. R. Glass

## INTRODUCTION

The principal short-term constraints on the utilization of coal reserves of the northcentral United States are the amount of environmental degradation that the American public is willing to sustain as the price for secure and abundant energy and the ability of scientists to forecast the amount and kinds of environmental impacts that will result from a given level of coal use.

Because of the rapid expansion of coal mining and coal-fired power generation in the northcentral great plains and because of the very limited knowledge and experience with the responses of the range resource to air pollution and other impacts of coal development, it is important to assess the potential responses of these ecosystems. These grasslands exhibit limited resilience to disturbance, and there is thus a potential for irreversible effects. It is not now possible to predict the long-term consequences of even the present level of coal production in the west (Lawton and McNeil, 1973; Library of Congress, 1975).

One of the primary concerns of the Colstrip, Montana, Coal-fired Power Plant Project (CFPPP) is the development of methods for the predictive assessment of impact. If this project is successful, the ability to judiciously plan regional power plant inventories and to select sites for new power production facilities will be greatly enhanced. Furthermore, if the nature and extent of specific impact can be predicted, steps to minimize or to ameliorate impacts may be possible. If the predictions are accepted, it should be simpler to resolve conflicts among parties concerned with the siting process and to arrive at constructive agreements that reduce the overall costs of power plant siting.

## PREDICTIVE ASSESSMENT OF IMPACT

Federal legislation requires the prediction of impact whether the capability exists or not. The National Environmental Policy Act of 1969 (NEPA) requires that an environmental impact statement (EIS) be filed for any project that requires federal approval. The EIS is to identify and describe potential environmental impacts of such projects and to evaluate their significance. NEPA, however, provides only limited guidelines for the conduct and preparation of the EIS. Consequently, most EISs provide few data that are useful to decision makers or that satisfy the intent of the law.

The problems of evaluating and forecasting environmental consequences and developing the technological methods to reduce or ameliorate impact are complex, and CFPPP contributions at this time are little more than embryonic. Both strip mining and coal combustion produce effects on living and non-living components of the environment that are extensive and diverse. While some effects may be ephemeral (acute toxicity of sulfur dioxide, fluoride, nitrogen oxides, etc.), others may prove to be insidious and long-lasting or irreversible (acid rainfall, long-term soil fertility decline, decreased energy flow, retrograde succession, etc.).

## AVAILABLE FORECASTING TOOLS

Forecasting tools that have been more or less routinely employed (e.g., guessing, extrapolation or rationalization of retrospective information, etc.) have had very limited or unknown success, because follow-up on predictions is infrequent. Those tools that have the greatest potential for predicting environmental impacts on terrestrial ecosystems (e.g., the use of controlled in situ field stressing with pollutants of concern combined with appropriate laboratory experiments; simulation models such as mass balance models; models of simple food webs; and diffusion models that employ data from the sites or regions of concern) are rarely employed.

Four basic types of predictive tools can be identified. These are qualitative techniques, retrospective analyses of data and trend extrapolation, process models, and empirical methods coupled to predictive models. Qualitative methods are based upon judgments and intuition that are logically arrived at in the absence of any satisfactory theory, direct experience or quantitative data relating to the problem. Such methods may lead to conceptual models that may indicate what data are needed and can generate hypotheses that can be tested. Qualitative methods include Delphi methods, panel (workshop) concensus, intuition and visionary forecast, and historical analysis. Occasionally, historical analogy (which is applied widely in EISs) proves useful for planning horizons of several years.

The use of scenarios, while not a predictive technique, should also be mentioned. Scenarios are hypothetical narratives that explore potential alternatives, usually those that seem reasonable or possible (worst case, best case). They are speculative, but can provide some insights into the range of possible future states of the system and can force consideration of options that might otherwise be overlooked.

Methods that employ retrospective data and trend analysis may be very effective where relevant data are fairly complete for one or more decades and when the functions of interest are noise-free and stable or follow an obvious trend. The basic assumption underlying these methods is that the observed patterns will continue into the future. Consequently, such methods tend to be better predictors of specific short-term futures than of long-term trends. Since such methods are based only upon time series analysis and extrapolation, the relationships of environmental variables to trends are unknown. As a result, predictions are highly site and time specific. A further serious drawback to the use of retrospective data is that it typically includes information that is not standardized, or is incomplete. Such data must be used selectively and this provides excellent opportunity for the introduction of social, economic or political bias.

Process models can be powerful predictive tools. While costly to develop, such models have a good potential for long-term prediction if they are based upon the results of appropriate field and laboratory experiments that are designed to evaluate the effects of random and non-random variables on the output.

The environmental residual technique has also been used in environmental impact assessment. This technique could be much improved by a more thorough assessment of impact than is usual. Undesirable effects (residuals) of power generation can be determined through a detailed knowledge of the power plant operations. One dimension of a two-dimensional matrix defines each residual. The other dimension lists management strategies. By filling out the cells or elements within this matrix, it is possible to optimize the management of the power plant based upon any one or all of the residuals of interest. Different management methods might prove to be most effective for different types of pollutants. For example, it is possible that $SO_x$ could best be managed by building a tall stack or by intermittent controls, while management of heavy metals might be best accomplished through the construction and operation of a baghouse. The detail of each management strategy relative to each residual would in turn be dependent upon engineering design and properties of the particular coal which was to be mined. The main difficulty is in relating the residuals to a detailed environmental impact assessment which can in turn feed back upon the initial design and site location of the power plant so as to minimize impacts.

For the environmental residuals method to be effectively applied, a detailed impact assessment with predictive capabilities is required. Residual allocation models have been linked to models that determine resource availability based on economics as well as geography and end uses of the particular energy activity from which residuals are generated (Federal Power Commission, in press). Briefly, this is accomplished through modeling that depends upon a knowledge of the resource being exploited, the technology being used, the chemical and other residuals which are produced by the technology, and the economics at each stage. These factors can be used to generate a matrix of residuals that can be related to impact on the environment. This in turn determines the management strategy for meeting air and water quality standards as well as socioeconomic criteria.

Controlled field exposures to selected pollutants, such as those being conducted by the CFPPP research team (described subsequently) can enable management to determine the exact relationship between the rate of exposure to a particular pollutant and the response of local biota. Such prediction of specific environmental impacts at a specific site could form the basis for more rational siting decisions (Woodwell, 1970).

## ECOLOGICAL CONCEPTUAL FRAMEWORK

A large and expanding body of theory and empirical information dealing with stress ecology serves as the basis for an ecological model of the effects of coal-fired power plant emissions on northern grassland ecosystems. A conceptualization of this is shown in Figure 1. The "target population" may be defined either taxonomically or functionally. An ecosystem functional model can be constructed by coupling the models of functionally important "target populations." The degree of resolution within the ecosystem model can be set by the definitions assigned to "target populations." By examining the response of the model to changes in air quality over time it is possible to characterize the "emergent" properties of the system.

The quality and magnitude of direct effects of pollutants on organismic function will undoubtedly vary among species and all higher taxonomic groups. This differential selective pressure provides the basis for indirect effects upon less sensitive biota and upon ecosystem structure and dynamics. Investigators in this project are interested in the relative sensitivities of species to both direct and indirect effects and the relative homeostatic and competitive capabilities of these species. The time lag (which may or may not be appreciable) between the occurrence of direct effects and the appearance of indirect effects and the degree of reversibility of these effects are also of interest.

A number of parameters and functions should be investigated as potential progenitors of systems-level indirect effects in northern plains grasslands. Effects on critical resources such as soil moisture, nutrients, solar radiation, and trace elements are likely to be very important.

Much of the dynamics of the northern plains grassland ecosystem appears to be regulated/controlled by the vagaries of the water cycle (Van Dyne, 1973). Particulate emissions can affect the formation and building of clouds by acting as cloud condensation nuclei or ice nuclei upon which water vapor condenses. The hot, dry air that accompanies these aerosols as they are swept into the base of clouds may also be important. Under certain conditions precipitation may be increased by the presence of particulate emissions. Under other circumstances precipitation may be reduced (Library of Congress, 1975). This could alter the amount of water to biological components of the ecosystem and could lead to major shifts in successional trends. Water stress may interact synergistically with potentially toxic air emissions, affecting the susceptibility of some system components to direct toxic effects.

By influencing the degree and nature of cloud formation, and by direct scattering and adsorption, particulate emissions may affect the quality and quantity of solar radiation impinging on the ecosystem. This could influence primary productivity selectively, by species, and thus serve as a major modifying force.

Certain gaseous emissions may influence the nutrient cycle. At low levels, $SO_2$ and $NO_x$ may serve as a source of plant nutrients. If portions of the system are nutrient-limited, these low level emissions could have a stimulatory effect

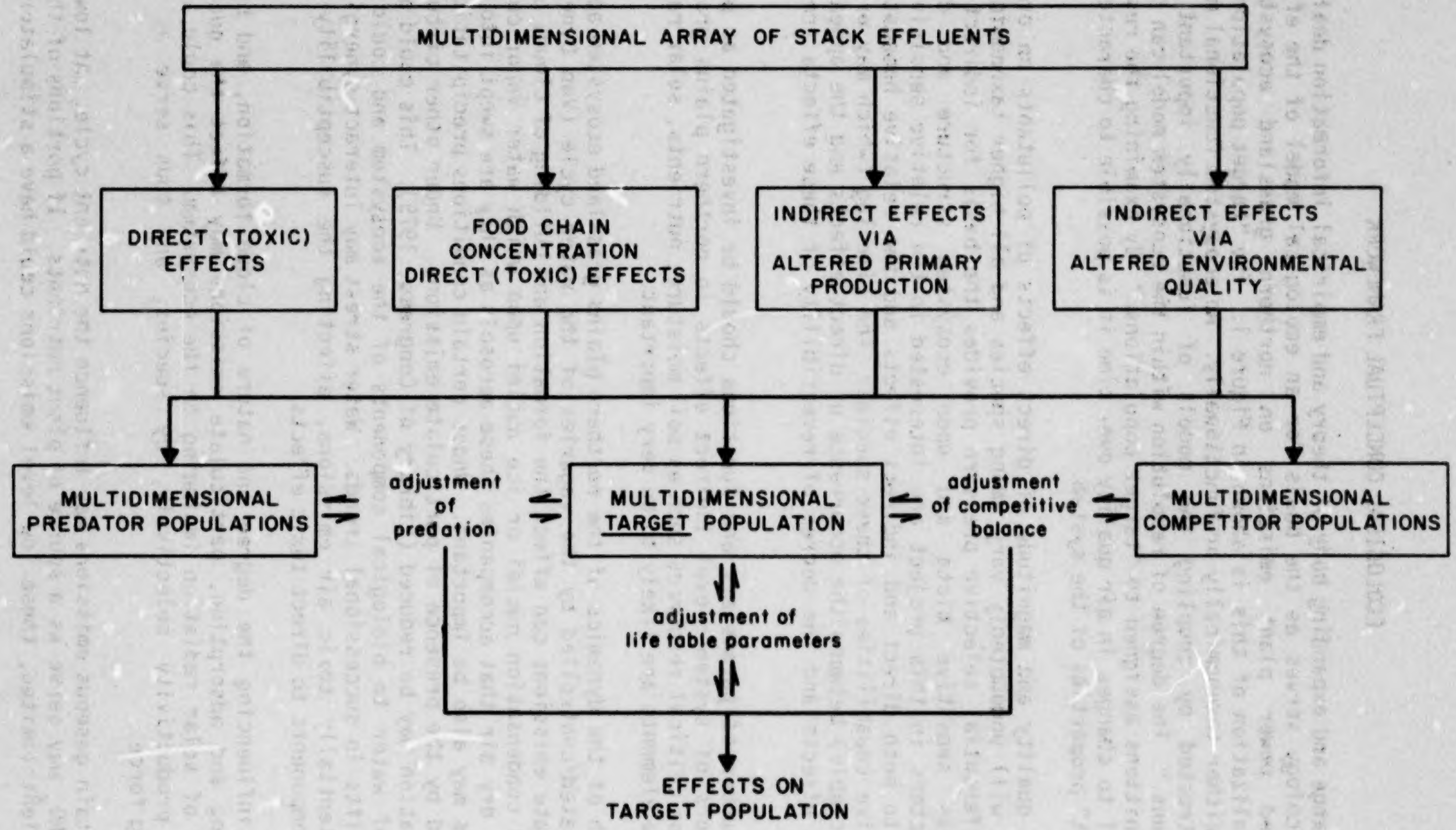
MULTIDIMENSIONAL ARRAY OF STACK EFFLUENTS
DIRECT (TOXIC) EFFECTS
FOOD CHAIN CONCENTRATION DIRECT (TOXIC) EFFECTS
INDIRECT EFFECTS VIA ALTERED PRIMARY PRODUCTION
INDIRECT EFFECTS VIA ALTERED ENVIRONMENTAL QUALITY
MULTIDIMENSIONAL PREDATOR POPULATIONS
adjustment of predation
MULTIDIMENSIONAL TARGET POPULATION
adjustment of competitive balance
MULTIDIMENSIONAL COMPETITOR POPULATIONS
adjustment of life table parameters
EFFECTS ON TARGET POPULATION

Figure 1. Autecological perspective of pollutant-ecosystem interaction.

on plant growth. At higher levels, such emissions begin to have direct toxic effects which may override the stimulatory effects of low level emissions.

When primary critical resources are affected, changes in physiological functions or population parameters of "key" species will quickly follow. These may include changes in growth rate, maturation time, reproductive rate, and rate of phenological development. Changes in populations that are of sufficient magnitude will alter community structure. Proportions of species may begin to change, new species may successfully invade. Of interest is how these structural changes affect community productivity and stability, and also the degree of reversibility of these changes.

## APPLICATION OF PREDICTIVE ASSESSMENT

There are nearly as many approaches to the selection of power plant sites as there are utilities in this country. The process is governed by many individual circumstances and a diversity of local and state laws and procedures. Further, the business concerns of the power company or utilities involved, value judgments by various decision makers, legislation, lobbies and the potential for adversary proceedings can influence all stages of the decision process.

In general, control of the location of power plants and attendant facilities resides with state governments. Policies differ among states and in some, comprehensive siting laws have been enacted. Montana, for example, has a planning mechanism that requires state certification. Plans for specific energy facilities must be submitted to the state ten years in advance of construction. The state evaluates the collective prospective impact of all facilities and certifies individual plants on the basis of this review. In many states, however, actual zoning, review, and certification of power plants is delegated to local governments; state review may not even be required. The individual company usually selects a site and seeks whatever zoning changes may be required.

The selection of sites for new plants appears often to be haphazard and is initially dictated by such factors as ownership or availability of lease lands and by engineering constraints. From an often large set of available sites, only a few will be subjected to detailed feasibility studies. Perhaps one to three are then evaluated according to NEPA guidelines to determine whether or not the proposed facility will have an acceptable level of environmental impact. The selection of appropriate sites can be very costly, and in recent years, a number of proposed facilities have been denied permits following the review of environmental impact statements. Often the utility has no clear idea until this time whether or not the proposed facility has an opportunity for approval.

The objective of this project is to develop a procedure that when applied will yield biological effects information and solutions that will contribute to the site selection decision. For the present, efforts are restricted to consideration of the biological effects of coal-fired power plant emissions on terrestrial ecosystems of the northern great plains with the Colstrip CFPP Project providing specific data and the first application of certain protocol elements. Within these constraints project investigators hope to develop methods to predict a set of futures and their range of probabilities and to utilize this information to influence the course of events so that undesirable impacts of CFPP operation may be ameliorated.

The prediction of environmental impact, however effective, is of no value to the siting process unless it is appropriately wedded to the constraints and needs of the decision making processes. Representatives from government regulatory agencies, producer and user industries, environmental groups and citizen groups must be included early in the protocol development process in order to define the policy and user constraints under which the protocol must be developed. Continuing consultation should be maintained with these policy groups throughout the process of protocol development.

## ELEMENTS OF A SITING PROTOCOL

The protocol will consist of a methodology to select suitable sites for construction of coal-fired power plants given several alternatives and a continuing post-siting methodology to ameliorate undesirable biological effects should they appear. Post-siting developments and continuing information inputs to the protocol should favor flexible or adaptive operational management of the working facilties. The chronology of information and action flow is displayed in Figure 2.

The annual patterns of concentrations of pollutants that would result from constructing a coal-fired power plant at each of the alternative sites could be predicted by a plume dispersion model or plume tracing studies.

A set of widely acceptable methods for gathering "baseline" information on the structure and dynamics of the terrestrial ecosystems at alternative sites is also needed. The pollution predictions and "baseline" ecosystems information will then serve as inputs to one or a series of biological response models whose outputs predict the biological effects of predicted pollutant concentrations on the ecosystem under consideration.

This information on potential biological effects on several alternative sites must be considered in conjunction with outputs from a socioeconomic and engineering protocol to arrive at a siting decision. In specifying outputs for the biological response models, provision must be made for interfacing with a socioeconomic segment. Ecological effects must be translated into predictive alternatives in man's relationship to the ecosystem (e.g., changes in agricultural productivity, changes in numbers of esthetic species, changes in number of recreational wildlife species, changes in ecosystem management practices).

Once a decision to site a plant has been made and construction is completed, the post-siting study is implemented. Data gathering is now directed to monitoring pollutant concentrations and biological effects in the vicinity of the power plant. These data are used to validate and improve the predictive models employed in the site-selection protocol. They will also provide the information base for adaptive management of the power plant. If pollution concentrations or biological effects reach unacceptable levels, government and/or management will have the necessary information to make rational modifications of plant operations.

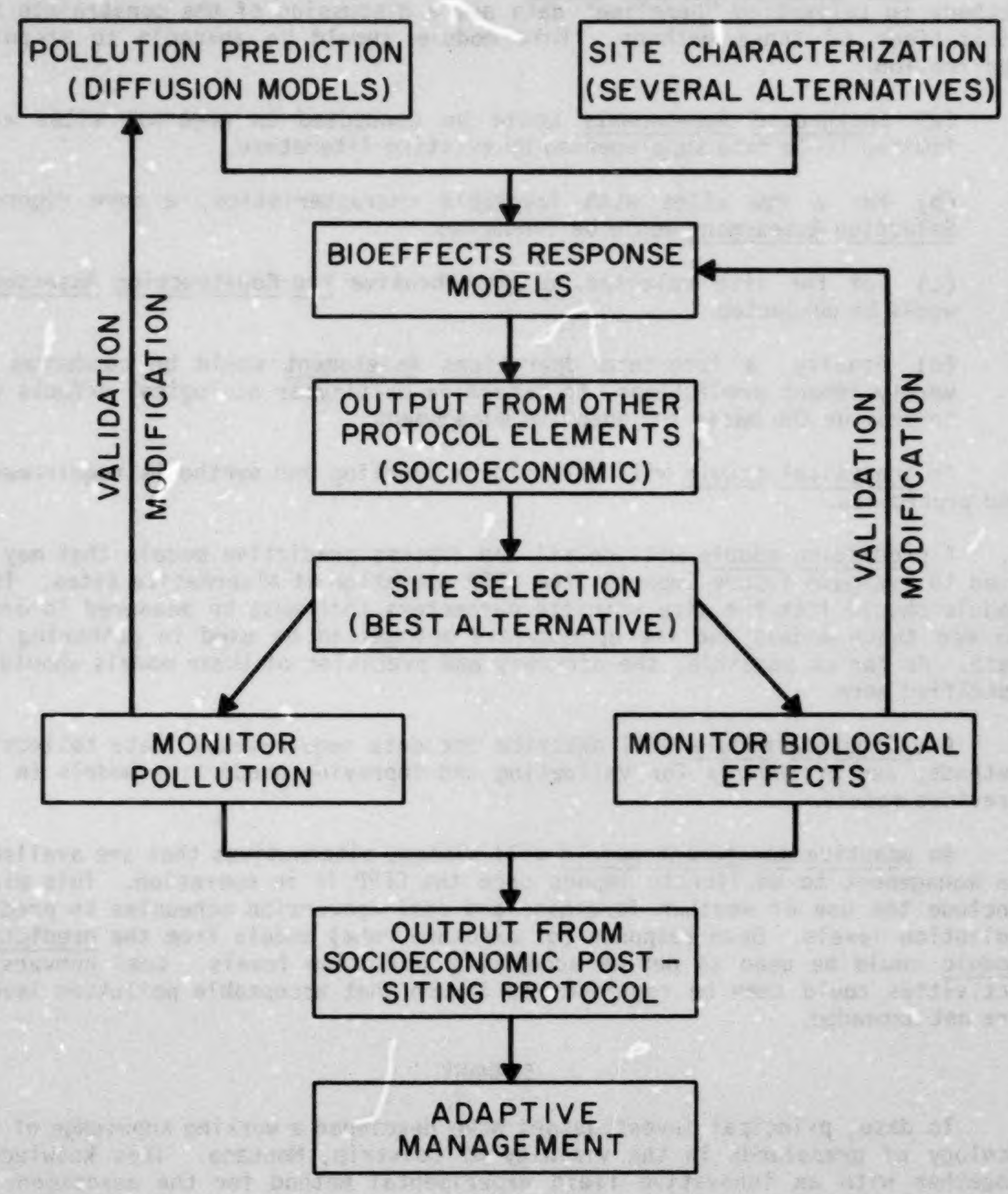
POLLUTION PREDICTION (DIFFUSION MODELS)
SITE CHARACTERIZATION (SEVERAL ALTERNATIVES)
BIOEFFECTS RESPONSE MODELS
OUTPUT FROM OTHER PROTOCOL ELEMENTS (SOCIOECONOMIC)
SITE SELECTION (BEST ALTERNATIVE)
MONITOR POLLUTION
MONITOR BIOLOGICAL EFFECTS
VALIDATION
MODIFICATION
VALIDATION
MODIFICATION
OUTPUT FROM SOCIOECONOMIC POST-SITING PROTOCOL
ADAPTIVE MANAGEMENT

**Figure 2. Generalized flow diagram of the ecological effects protocol.**

The final bioeffects protocol as presently conceived will consist of five modules.

1. A site characterization module will include a discussion of preferred methods in collecting "baseline" data and a discussion of the constraints and limitations of these methods. This module should be amenable to stepwise application.

   (a) Background Assessments would be conducted on numerous sites with limited field data supplemented by existing literature.

   (b) For a few sites with favorable characteristics, a more rigorous Selection Assessment would be conducted.

   (c) For the site selected, a comprehensive Pre-Construction Assessment would be conducted.

   (d) Finally, a long-term Operations Assessment would be conducted to verify impact predictions, to determine multi-year ecological effects and to provide the basis for adaptive management.

2. An analytical module will define data handling and synthesis requirements and procedures.

3. A predictive module will detail and discuss predictive models that may be used to estimate future impacts from CFPP operation at alternative sites. This module should list the site specific parameters that must be measured in order to use these models and the appropriate methods to be used in gathering the data. As far as possible, the accuracy and precision of these models should be specified here.

4. A validation module will describe the data requirements, data collection methods, and procedures for validating and improving predictive models in the previous module.

5. An adaptive management module will discuss alternatives that are available to management to ameliorate impact once the CFPP is in operation. This might include the use of weather forecasts and coal conversion schedules to predict pollution levels. Dose-response (or exposure rate) models from the predictive module could be used to define acceptable pollution levels. Coal conversion activities could then be regulated to insure that acceptable pollution levels are not exceeded.

## SUMMARY

To date, principal investigators have developed a working knowledge of the ecology of grasslands in the vicinity of Colstrip, Montana. This knowledge, together with an innovative field experimental method for the assessment of ecological impact of air pollution, is slowly providing the information required to develop predictive capability. Such a capability will allow managers to plan and to control the siting process, to modulate (or ameliorate) impacts through adaptive management and to anticipate the possibility and needs for rehabilitation. Improved management should be effected not only through more effective and efficient site-selection procedures, but also through evolution of the siting protocol (update and validation) and the adaptive management of power facilities through all phases of their life cycle.

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# COLSTRIP STUDIES

## SECTION 1

# EFFECTS OF $SO_2$ AND OTHER COAL-FIRING PLANT EMISSIONS ON PRODUCER, INVERTEBRATE CONSUMER, AND DECOMPOSER STRUCTURE AND FUNCTION IN THE VICINITY OF COLSTRIP, MONTANA

by

W. K. Lauenroth, J. L. Dodd, R. K. Heitschmidt and J. W. Leetham

## INTRODUCTION

Within the next 25 years the northern Great Plains of the United States will be subjected to air pollution from energy development. The air pollution will come from the conversion of coal to electricity through steam generating power plants. Most of the coal in the northern Great Plains lies under native grassland and much of it will be converted to electricity near the open mines. Sulfur dioxide and other air pollutants from the conversion process will be released onto the adjacent native grasslands.

The objectives of this study are to look at the effect of air pollution on primary producers, arthropod consumers, and decomposition processes. The study has three aspects. At Colstrip, this consists of baseline monitoring of decomposition processes and productivity and seasonal biomass dynamics of primary producers and arthropods at four different sites that range from 11 to 18 km east and southeast of the power plant. Baseline monitoring began in 1974 and will continue over the next several years to determine what, if any, influence air pollution has on these grasslands.

## COLSTRIP MONITORING SITES

The 1975 growing season marked the end of a baseline data collection phase for the Colstrip Power Plant Project. Unit I of the generating complex at Colstrip began operation in the fall of 1975 and the growing season of 1976 represents the first year of data collection under the influence of the power plant operation. An important task now to be completed with respect to the baseline data is to compare and contrast the two years of baseline data collection, 1974 and 1975, with respect to abiotic conditions in an attempt to relate differences in ecosystem response to differences in abiotic conditions during the two years. The objective of this report is to present the 1975 data and make some initial comparisons with the data collected in 1974.

## ABIOTIC DATA

The collection of abiotic data at the Colstrip sites was not begun until the growing season of 1975. The U.S. Department of Commerce weather data for southeastern Montana can be used to make general comparisons between the two years. Precipitation data for 1974 and 1975 are recorded in Table 1.1. Data for 1974 are from the U.S. Department of Commerce Weather Station at Colstrip and 1975 data were collected at the four monitoring sites. The Colstrip Station (USDC) apparently was not operated in 1975 and data are not available to make comparisons between 1974 and 1975 to see how these data compare with data collected at the monitoring sites. Total precipitation for the May to September period was 228 mm in 1974 and 261 mm average for the four sites in 1975. More important than the differences in total precipitation were the differences in the May and June precipitation. In 1974, 121 mm of precipitation were recorded during May and June and in 1975, 221 mm were measured.

The average monthly temperatures in Table 1.2 are presented for general comparison of the growing seasons during 1974 and 1975 and do not necessarily represent average monthly temperatures for the Colstrip sites. The major differences between the two years included a much cooler April, a slightly warmer May, and a cooler June.

Soil water data collected at the Colstrip sites in 1975 reflected the high precipitation during May and June (Table 1.3). Soil water to a depth of 105 cm remained above 20 cm on all sites through 30 June. This was followed by a rapid depletion during the first two weeks of July and a subsequent gradual depletion throughout the remainder of the growing season. No major differences were evident among sites except for small differences in total soil water observed throughout the period. The Hay Coulee and Kluver East sites apparently have the highest water-holding capacity and the Kluver North site the lowest. Soil water at the end of the growing season was highest at the Kluver East site and lowest at the Kluver North site and averaged approximately 10 cm to a depth of 105 cm for all sites.

Soil surveys of the Colstrip sites were made in 1975. Although physical and chemical descriptions are incomplete at this time, profile descriptions for Hay Coulee, Kluver West, and Kluver East are included in the Appendix.

## PLANT PHENOLOGY

Phenological observations were made on a weekly basis using a 14-stage phenological classification (Table 1.4) during the 1975 season. Fourteen species were observed and represented a range from Hood's phlox which was flowering at the time of the first observation to fringed sagewort which had just begun flowering at the time of the last observation in mid-September (Tables 1.5 and 1.6). Few differences in the timing of phenological events were among sites with stage 14, drought-induced dormancy, being the most variable of the stages and presumably reflecting differences in soil water among sites.

TABLE 1.1. GROWING SEASON PRECIPITATION (mm) FOR THE COLSTRIP SITES DURING THE GROWING SEASON IN 1974 AND 1975.

| Month | Colstrip 1974 | Colstrip sites 1975 | | | | |
|---|---|---|---|---|---|---|
| | | Hay Coulee | Kluver West | Kluver North | Kluver East | Average |
| May | 94 | 121 | 121 | 120 | 113 | 119 |
| June | 27 | 94 | 93 | 116 | 104 | 102 |
| July | 50 | 17 | 23 | 13 | 14 | 17 |
| Aug. | 28 | 16 | 21 | 19 | 15 | 18 |
| Sept. | 29 | 5 | 5 | 5 | 6 | 5 |
| Total | 228 | 253 | 263 | 273 | 252 | 261 |

TABLE 1.2. AVERAGE MONTHLY TEMPERATURE (°F) FOR SOUTHERN MONTANA DURING 1974 AND 1975.

| Month | Southeastern Montana average | |
|---|---|---|
| | 1974 | 1975 |
| April | 46.5 | 38.4 |
| May | 50.8 | 52.5 |
| June | 65.2 | 61.3 |
| July | 73.9 | 73.0 |
| Aug. | 63.7 | 67.1 |
| Sept. | 55.0 | 56.2 |

TABLE 1.3. SEASONAL DYNAMICS OF SOIL WATER (cm), COLSTRIP STUDY SITES, 1975.

| Treatment | Depth (cm) | 3 May | 14 May | 4 June | 19 June | 30 June | 14 July | 29 July | 9 August | 25 August | 9 September |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Hay | 0-15 | 3.73 ± .19 | 2.95 ± .24 | 1.95 ± .10 | 4.13 ± .18 | 1.99 ± .21 | 0.99 ± .09 | 1.33 ± .09 | 0.80 ± .14 | 0.59 ± .04 | 0.69 ± .05 |
| Coulee | 15-30 | 4.04 ± .37 | 4.00 ± .28 | 2.33 ± .55 | 3.54 ± .49 | 2.70 ± .41 | 1.42 ± .22 | 1.55 ± .18 | 1.09 ± .25 | 1.10 ± .23 | 1.21 ± .19 |
|  | 30-45 | 4.54 ± .06 | 4.39 ± .34 | 3.52 ± .34 | 3.36 ± .32 | 3.23 ± .30 | 1.83 ± .18 | 1.92 ± .12 | 1.44 ± .26 | 1.51 ± .25 | 1.65 ± .46 |
|  | 45-60 | 4.79 ± .47 | 4.29 ± .36 | 3.97 ± .04 | 3.28 ± .33 | 3.19 ± .33 | 2.03 ± .18 | 2.44 ± .16 | 1.96 ± .84 | 1.56 ± .12 | 1.41 ± .24 |
|  | 60-75 | 4.17 ± .31 | 3.68 ± .63 | 3.20 ± .15 | 3.44 ± .15 | 2.95 ± .22 | 2.22 ± .28 | 2.67 ± .49 | 2.27 ± .36 | 1.80 ± .19 | 1.84 ± .33 |
|  | 75-90 | 3.34 ± .35 | 3.43 ± .46 | 3.09 ± .50 | 3.45 ± .39 | 3.21 ± .40 | 2.32 ± .40 | 2.88 ± .66 | 2.72 ± .16 | 1.85 ± .21 | 1.53 ± .14 |
|  | 90-105 | 1.73 ± .20 | 3.30 ± .26 | 3.16 ± .35 | 3.28 ± .55 | 2.93 ± .34 | 2.53 ± .45 | 2.23 ± .12 | 2.02 ± .18 | 2.07 ± .27 | 2.15 ± .33 |
| TOTAL |  | 26.35 | 26.04 | 21.22 | 24.49 | 20.20 | 13.43 | 15.01 | 12.30 | 10.49 | 10.48 |
| Kluver | 0-15 | 3.36 ± .11 | 2.96 ± .29 | 1.98 ± .19 | 2.03 ± .07 | 2.23 ± .19 | 0.72 ± .04 | 0.96 ± .13 | 0.59 ± .04 | 0.73 ± .11 | 0.68 ± .04 |
| West | 15-30 | 3.34 ± .05 | 3.27 ± .03 | 2.78 ± .23 | 2.70 ± .20 | 2.78 ± .78 | 1.21 ± .08 | 1.51 ± .30 | 1.00 ± .13 | 0.95 ± .09 | 1.28 ± .37 |
|  | 30-45 | 3.77 ± .03 | 3.01 ± .56 | 3.12 ± .20 | 2.56 ± .08 | 3.16 ± .44 | 1.50 ± .04 | 2.17 ± .31 | 1.07 ± .13 | 0.94 ± .01 | 1.02 ± .10 |
|  | 45-60 | 3.90 ± .16 | 2.83 ± .78 | 2.87 ± .15 | 3.46 ± .61 | 3.31 ± .51 | 1.67 ± .13 | 2.53 ± .23 | 1.14 ± .07 | 1.10 ± .13 | 1.57 ± .41 |
|  | 60-75 | 3.31 ± .08 | 3.39 ± .18 | 3.23 ± .52 | 3.44 ± .09 | 3.14 ± .42 | 2.64 ± .82 | 2.56 ± .12 | 1.50 ± .37 | 1.45 ± .18 | 1.74 ± .54 |
|  | 75-90 | 3.70 ± .29 | 4.23 ± .20 | 3.02 ± .40 | 2.76 ± .23 | 3.29 ± .36 | 2.67 ± .77 | 2.40 ± .34 | 1.84 ± .64 | 2.70 ± .22 | 2.45 ± .77 |
|  | 90-105 | 3.25 ± .33 | 4.07 ± .25 | 3.06 ± .39 | 3.54 ± .63 | 3.40 ± .64 | 2.49 ± .53 | 2.60 ± .43 | 2.13 ± .18 | 2.38 ± .75 | 1.81 ± .35 |
| TOTAL |  | 24.63 | 23.77 | 20.07 | 20.49 | 21.31 | 12.91 | 14.73 | 9.27 | 10.25 | 10.56 |
| Kluver | 0-15 | 3.04 ± .20 | 2.30 ± .03 | 1.93 ± .06 | 2.28 ± .13 | 2.40 ± .50 | 1.19 ± .19 | 0.94 ± .08 | 0.60 ± .03 | 0.73 ± .09 | 0.63 ± .13 |
| North | 15-30 | 3.39 ± .20 | 2.41 ± .63 | 2.50 ± .31 | 3.16 ± .20 | 2.99 ± .47 | 1.61 ± .26 | 1.44 ± .20 | 0.94 ± .10 | 1.13 ± .06 | 1.12 ± .25 |
|  | 30-45 | 3.83 ± .20 | 3.47 ± .07 | 3.13 ± .39 | 3.25 ± .19 | 3.15 ± .08 | 2.00 ± .21 | 1.67 ± .14 | 1.06 ± .11 | 1.24 ± .07 | 1.30 ± .21 |
|  | 45-60 | 4.72 ± .92 | 3.46 ± .13 | 5.33 ± 1.80 | 3.44 ± .43 | 3.57 ± .03 | 2.55 ± .40 | 1.93 ± .27 | 1.24 ± .11 | 1.67 ± .31 | 1.01 ± .25 |
|  | 60-75 | 3.63 ± .14 | 3.56 ± .09 | 2.97 ± .53 | 3.30 ± .46 | 3.44 ± .18 | 2.54 ± .54 | 2.05 ± .27 | 1.20 ± .10 | 1.63 ± .36 | 1.32 ± .30 |
|  | 75-90 | 2.91 ± .34 | 3.66 ± .26 | 3.50 ± .31 | 3.26 ± .38 | 3.46 ± .27 | 2.54 ± .55 | 2.36 ± .16 | 1.53 ± .19 | 1.83 ± .56 | 1.55 ± .47 |
|  | 90-105 | 2.42 ± .14 | 3.16 ± .23 | 3.60 ± .30 | 3.43 ± .46 | 3.42 ± .12 | 2.93 ± .42 | 2.70 ± .26 | 2.09 ± .37 | 2.21 ± .52 | 1.81 ± .64 |
| TOTAL |  | 23.93 | 22.03 | 22.96 | 22.12 | 22.43 | 15.36 | 13.09 | 8.66 | 10.43 | 8.73 |
| Kluver | 0-15 | 3.52 ± .07 | 2.97 ± .26 | 2.21 ± .13 | 2.76 ± .21 | 2.26 ± .16 | 1.22 ± .16 | 0.88 ± .16 | 0.99 ± .37 | 0.78 ± .11 | 0.67 ± .15 |
| East | 15-30 | 4.16 ± .42 | 3.61 ± .69 | 3.57 ± .33 | 3.46 ± .40 | 2.66 ± .31 | 1.81 ± .30 | 1.34 ± .22 | 1.16 ± .12 | 1.12 ± .11 | 1.16 ± .10 |
|  | 30-45 | 4.36 ± .27 | 4.15 ± .32 | 3.22 ± .19 | 3.15 ± .63 | 2.87 ± .16 | 2.14 ± .09 | 1.39 ± .17 | 1.19 ± .11 | 1.77 ± .66 | 1.35 ± .12 |
|  | 45-60 | 4.05 ± .11 | 4.07 ± .23 | 3.25 ± .17 | 3.47 ± .10 | 3.02 ± .07 | 2.19 ± .22 | 1.60 ± .13 | 1.69 ± .26 | 1.28 ± .06 | 1.27 ± .09 |
|  | 60-75 | 3.99 ± .19 | 3.99 ± .25 | 3.14 ± .65 | 3.42 ± .27 | 3.43 ± .66 | 2.60 ± .21 | 1.92 ± .35 | 2.07 ± .09 | 1.84 ± .17 | 1.91 ± .13 |
|  | 75-90 | 3.51 ± .48 | 4.35 ± .16 | 3.65 ± .08 | 3.37 ± .33 | 3.14 ± .31 | 2.64 ± .26 | 2.66 ± .29 | 2.29 ± .21 | 2.35 ± .36 | 2.28 ± .06 |
|  | 90-105 | 2.01 ± .71 | 4.11 ± .28 | 3.37 ± .28 | 3.44 ± .32 | 2.96 ± .09 | 2.66 ± .20 | 2.94 ± .34 | 2.90 ± .47 | 2.47 ± .51 | 2.62 ± .34 |
| TOTAL |  | 25.61 | 27.24 | 22.42 | 23.07 | 20.36 | 15.26 | 12.74 | 12.29 | 11.61 | 11.26 |

TABLE 1.4. PHENOLOGICAL STAGES USED DURING THE 1975 GROWING SEASON.

| Phenological stages |
|---|
| 1. Winter dormancy |
| 2. First visible growth |
| 3. First leaves fully expanded |
| 4. Middle leaves fully visible |
| 5. Middle leaves fully expanded |
| 6. Late leaves fully expanded |
| 7. First floral buds |
| 8. Mature floral buds |
| 9. Floral buds and open flowers |
| 10. Floral buds, open flowers, and ripening fruit |
| 11. Buds, flowers, and green and ripe fruit |
| 12. Buds, flowers, green and ripe fruit, and seeds |
| 13. Green and ripe fruit, and dispersing seeds |
| 14. Dispersing seeds and drought-induced dormancy |

TABLE 1.5. PHENOLOGY OF MAJOR FORB SPECIES, COLSTRIP STUDY SITES, 1975 (A = Hay Coulee, B = Kluver West, C = Kluver North, D = Kluver East).

| | *Phlox hoodii* | | | | *Tragopogon dubius* | | | | *Hedeoma hispida* | | | | *Sphaeralcea coccinea* | | | | *Plantago patagonia* | | | | *Psoralea argophylla* | | | | *Artemisia frigida* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Date | A | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D |
| 27 May | 11 | 11 | 11 | 11 | 5 | 5 | 5 | 5 | 4 | | | | | 4 | 4 | | | 2 | | | | | 3 | 2 | 4 | 4 | 4 | 3 |
| 2 June | 13 | 13 | 13 | 12 | 7 | 7 | 7 | 7 | 4 | | | | | 5 | 5 | | | 2 | | | | 4 | 3 | 3 | 4 | 4 | 4 | 4 |
| 11 June | 14 | 14 | 13 | | 8 | 7 | 7 | | 4 | | | | | 5 | 5 | | | | | | 3 | 4 | 4 | | 5 | 4 | 4 | 4 |
| 18 June | | | | 14 | 9 | 8 | 9 | 9 | 8 | | | | | 7 | | | | | | | | 7 | 7 | 7 | 5 | 4 | 4 | 4 |
| 23 June | | | 14 | | 9 | 9 | 11 | 10 | 9 | | | | | 8 | 9 | | | 8 | | | | 7 | | 7 | 5 | 4 | 4 | 4 |
| 30 June | | | | | 11 | 10 | 11 | 10 | 10 | 10 | 10 | 10 | 10 | 9 | 10 | | 10 | 9 | 9 | 9 | | 8 | 8 | 8 | 5 | 4 | 4 | 4 |
| 8 July | | | | | 13 | 13 | 13 | 13 | 12 | 12 | 12 | 10 | | 11 | 10 | | 10 | 10 | 10 | 9 | | 10 | 10 | 9 | 5 | 4 | 4 | 5 |
| 14 July | | | | | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 12 | 13 | 11 | 10 | | 10 | 10 | 10 | 10 | | 10 | 10 | 10 | 5 | 5 | 5 | 5 |
| 22 July | | | | | 13 | 14 | 14 | 14 | 13 | 13 | 13 | 13 | 13 | 11 | 12 | | 13 | 13 | 12 | 12 | | 10 | 10 | 10 | 6 | 6 | 6 | 5 |
| 28 July | | | | | 13 | | | | 14 | 14 | 14 | 14 | 13 | 13 | 12 | | 13 | 13 | 13 | 13 | | 10 | 10 | 10 | 6 | 6 | 6 | 6 |
| 3 Aug. | | | | | 14 | | | | | | | | 13 | 13 | 13 | | 13 | 13 | 13 | 13 | | 13 | 13 | 13 | 6 | 6 | 6 | 6 |
| 10 Aug. | | | | | | | | | | | | | | 13 | 13 | | 14 | 13 | 13 | 13 | | 13 | 13 | 13 | 6 | 6 | 6 | 6 |
| 18 Aug. | | | | | | | | | | | | | 14 | 14 | 14 | | | 14 | 14 | 14 | | 13 | | 13 | 7 | 7 | 7 | 7 |
| 26 Aug. | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 1 Sept. | | | | | | | | | | | | | | | | | | | | | | | | 13 | 9 | 8 | 9 | 9 |
| 8 Sept. | | | | | | | | | | | | | | | | | | | | | | | | 14 | 9 | 8 | 9 | 9 |
| 14 Sept. | | | | | | | | | | | | | | | | | | | | | | | | | 9 | 9 | 9 | 9 |

TABLE 1.6. PHENOLOGY OF MAJOR GRASS SPECIES, COLSTRIP STUDY SITES, 1975 (A = Hay Coulee, B = Kluver West, C = Kluver North, D = Kluver East).

| | *Poa secunda* | | | | *Stipa comata* | | | | *Bromus japonicus* | | | | *Koeleria cristata* | | | | *Aristida longiseta* | | | | *Bouteloua gracilis* | | | | *Agropyron smithii* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Date | A | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D |
| 27 May | | | | | | | | | 7 | 7 | 7 | 7 | | | | | | | 3 | | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| 2 June | | | | | | | | | 8 | 8 | 8 | 8 | 8 | | | | | | 4 | | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| 11 June | 9 | | | | | | | | 8 | 8 | 8 | | 8 | | | | | | 4 | | 4 | 4 | 4 | | 4 | 4 | 4 | |
| 19 June | 9 | | | | | 8 | 8 | 9 | 9 | 8 | 8 | 9 | 9 | | 9 | | | | 7 | | 4 | 4 | 4 | 5 | 6 | 5 | 6 | 7 |
| 23 June | 10 | | | | | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | | | 7 | | 5 | 4 | 4 | 5 | 9 | 6 | 7 | 7 |
| 30 June | 10 | 10 | 10 | 9 | | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | | 8 | 8 | | 5 | 5 | 5 | 5 | 9 | 8 | 9 | 8 |
| 8 July | 12 | 12 | 12 | 11 | 10 | 11 | 11 | 10 | 10 | 9 | 9 | 10 | 9 | 9 | 9 | 11 | | 8 | 8 | | 7 | 6 | 6 | 7 | 10 | 9 | 9 | 9 |
| 14 July | 13 | 13 | 13 | 12 | 13 | 13 | 13 | 13 | 10 | 9 | 10 | 12 | 11 | 12 | 12 | 12 | | 8 | 8 | | 9 | 6 | 8 | 8 | 10 | 9 | 10 | 10 |
| 22 July | 14 | 14 | 14 | 13 | 14 | 13 | 13 | 13 | 13 | 12 | 13 | 13 | 13 | 13 | 13 | | | 10 | 10 | | 9 | 8 | 9 | 9 | 10 | 9 | 10 | 10 |
| 28 July | | | | 13 | | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | | 11 | 10 | | 10 | 10 | 10 | 10 | 11 | 10 | 11 | 11 |
| 3 August | | | | 14 | | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 13 | 13 | 13 | 13 | | 13 | 13 | | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 |
| 10 August | | | | | | | | | | | | | 13 | 13 | 13 | 13 | | 13 | 13 | | 12 | 12 | 12 | 12 | 11 | 11 | 12 | 11 |
| 18 August | | | | | | | | | | | | | 14 | 14 | 14 | 14 | | 13 | 14 | | 13 | 13 | 13 | 13 | 12 | 12 | 12 | 12 |
| 26 August | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 1 September | | | | | | | | | | | | | | | | | | | | | | 13 | 13 | | 13 | 13 | 13 | 13 |
| 8 September | | | | | | | | | | | | | | | | | | 14 | | | | 14 | 14 | | 13 | 13 | 13 | 13 |
| 14 September | | | | | | | | | | | | | | | | | | | | | | | | | 13 | 13 | 13 | 13 |

## ABOVEGROUND BIOMASS DYNAMICS

Figure 1.1 presents seasonal dynamics of total aboveground biomass for the four Colstrip sites during 1974 and 1975. Represented here are current live, recent dead (material that died during growing season of measurement) and old dead (material that died prior to growing season of measurement) biomass. The immediate differences observable on this figure are a larger amount of current live biomass in 1975 and the slight delay in the recording of the peak in current live biomass. Another difference between years, although not an unexpected one, is the larger amount of old dead biomass recorded in the spring of 1975 compared to 1974. This is a direct result of the exclusion of cattle by fencing and points to a problem that must be addressed in analyzing the baseline data. We must be able to sort out differences between the two years both in terms of differences in abiotic regimes and differences resulting from fencing. The Kluver East site which showed the largest increase in current live biomass between 1974 and 1975 also had the largest response to fencing. Although we have not looked at the data in terms of species composition, we expect that the effects of fencing on this site will be larger than on the remaining sites.

Figure 1.2 compares the current season production of cool season grasses, the most important functional group on the study sites, between 1974 and 1975. Again, the major differences are the larger absolute amount of current season production during 1975 and the slight delay in peak production. In 1974, peak production was recorded on approximately 15 July 1975. Although there was an increase in the production of cool season grasses in 1975, the relative position of each site remained constant for both years. The greatest amount of cool season grasses was found on the Kluver West site in both years and the least amount of cool season grass production was recorded for the Kluver North site for both seasons.

Figures 1.3 and 1.4 present current season's production for western wheatgrass and needle-and-thread grass, the two major contributors to the cool season grass group. Similar trends of greater production can be seen for both species during 1975 than during 1974. Western wheat-grass showed much less fluctuation in the measurement of current season's production from date to date in 1975 than in 1974. Needle-and-thread grass productivity was the greatest on the Kluver West site and not measurable for the Hay Coulee site. The Kluver East site had the least amount of needle-and-thread grass productivity in both 1974 and 1975.

Cool season forb productivity averaged approximately 10 g/m$^2$ for all sites in 1974 (Figure 1.5). It was only slightly higher (approximately 15 g/m$^2$) in 1975. The Kluver North site had the largest productivity of cool season forbs in both years and the Kluver East site the least amount of cool season forb production.

Half-shrub productivity was also greater in 1975 than in 1974 (Figure 1.6). The abrupt drop in half-shrub biomass recorded at the beginning of July in 1974 was not present in 1975. The Kluver East site had the greatest productivity of half-shrubs in 1975 while the Kluver North site had the greatest productivity in 1974. This again is an indication of the changes that are occurring in the Kluver East site, presumably as a result of fencing.

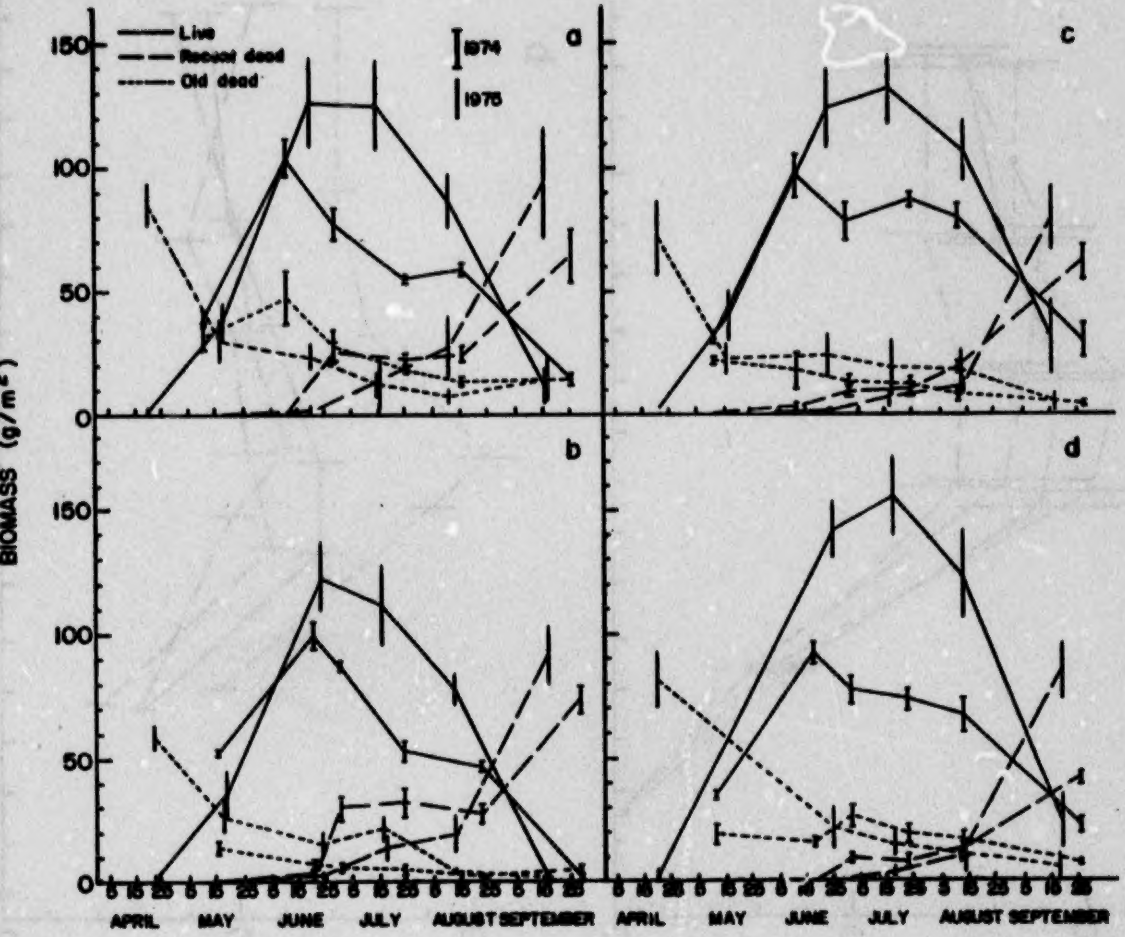


Figure 1.1. Comparison of aboveground biomass dynamics during the 1974 and 1975 growing seasons at the four Colstrip sites: (a) Hay Coulee, (b) Kluver West, (c) Kluver North, and (d) Kluver East.

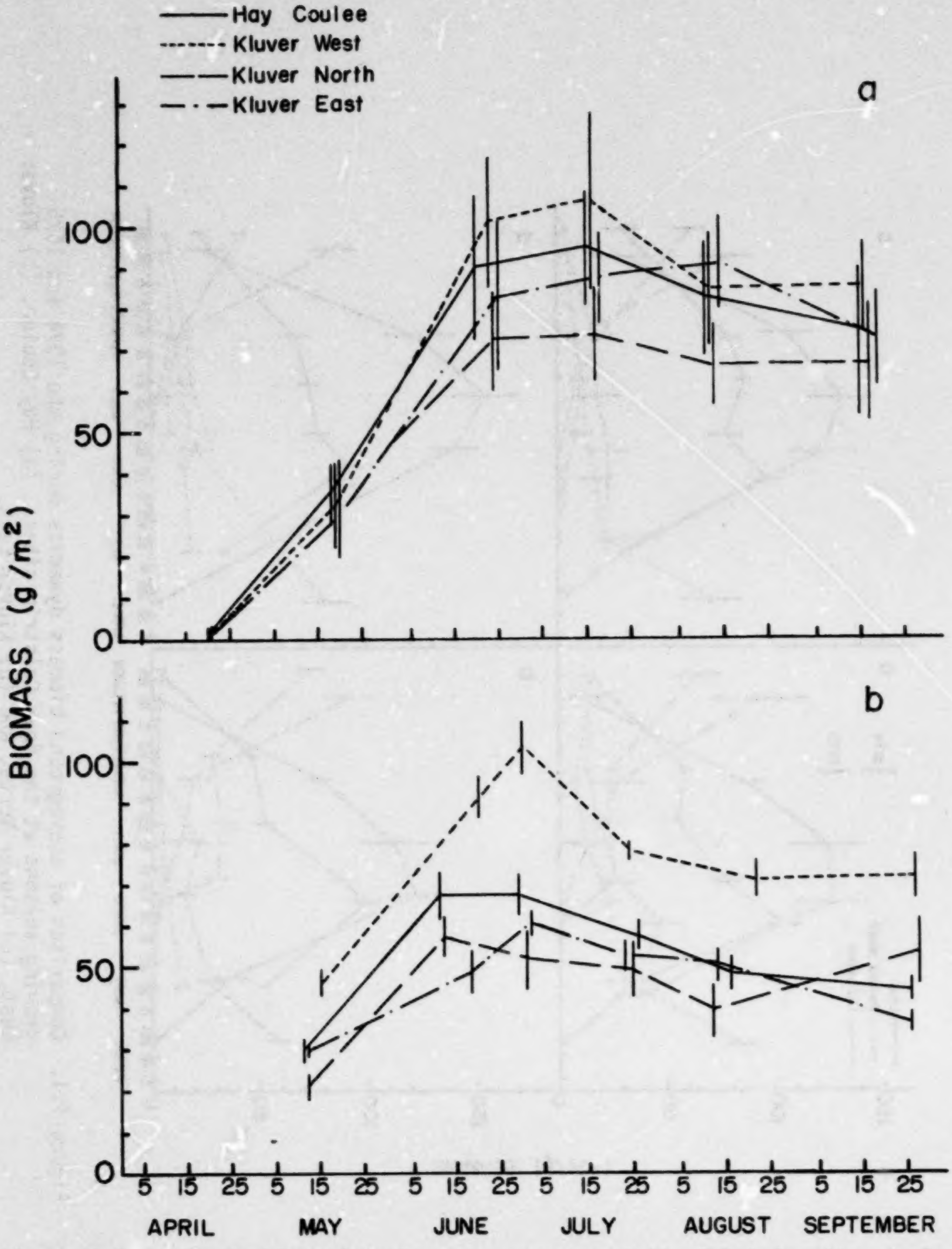


Figure 1.2. Production of cool season grasses at the Colstrip sites during the 1974 (b) and 1975 (a) growing seasons.

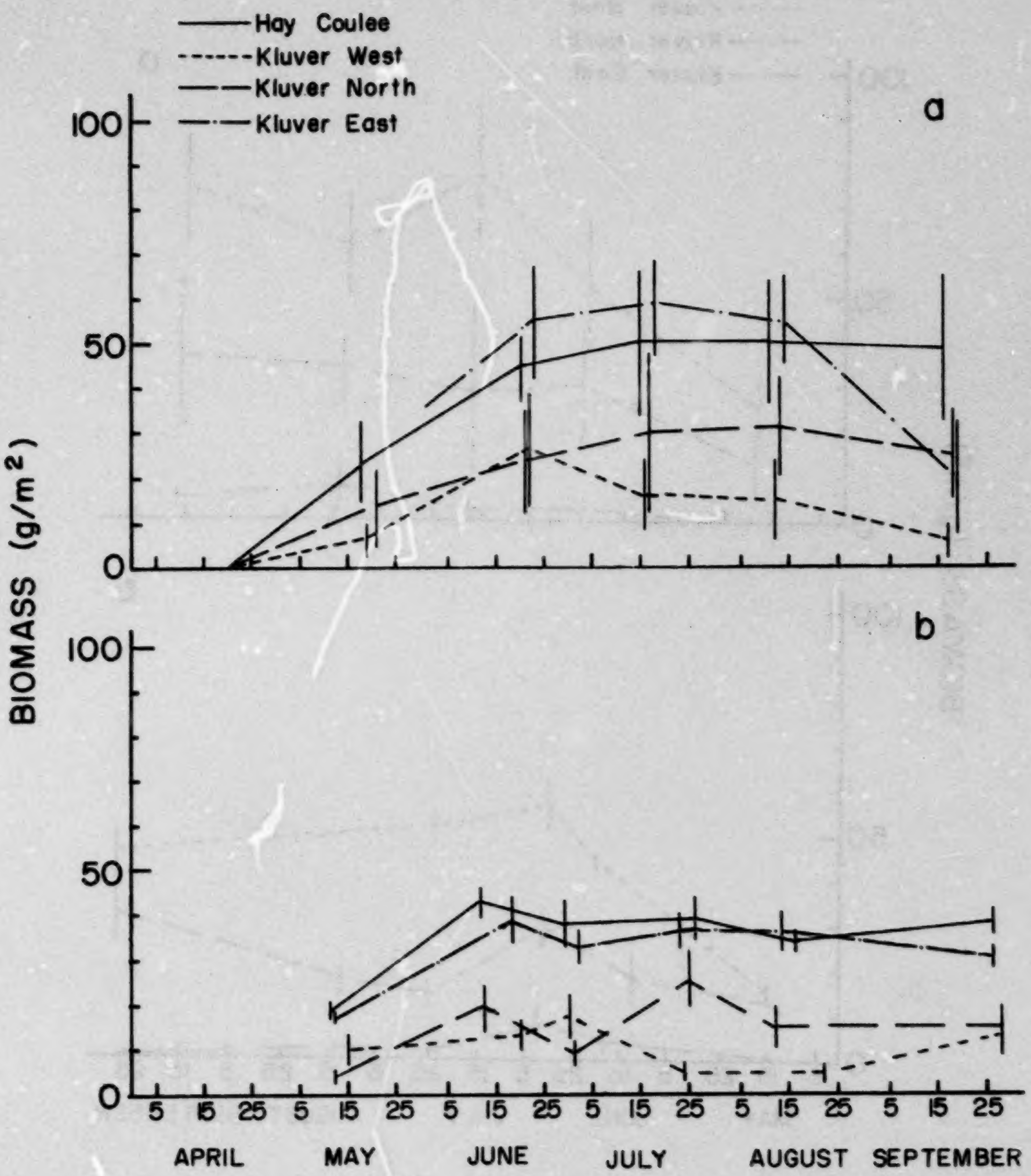


Figure 1.3, Production of western wheatgrass at the Colstrip sites during the 1974 (b) and 1975 (a) growing seasons.

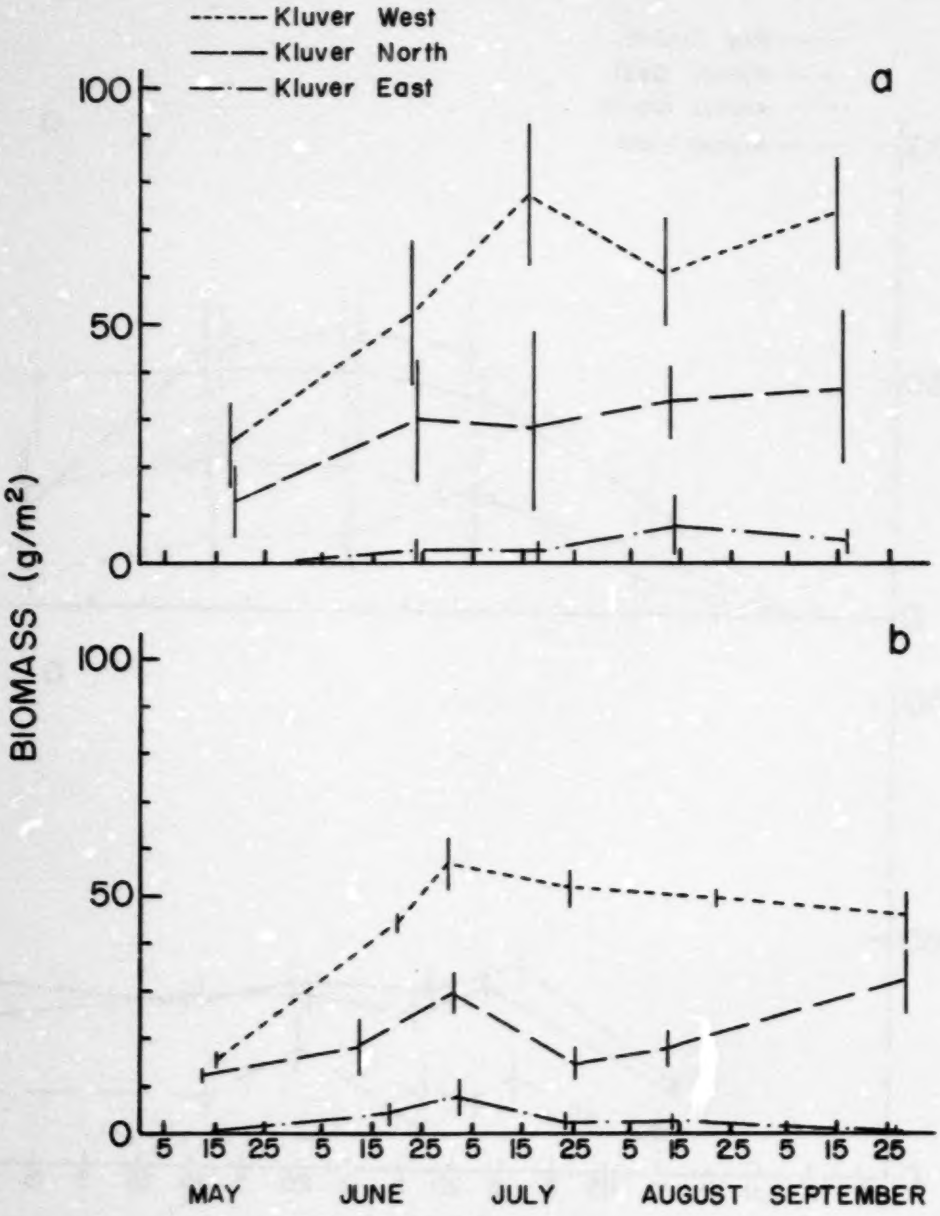


Figure 1.4. Production of needle-and-thread grass at the Colstrip sites during the 1974 (b) and 1975 (a) growing seasons.

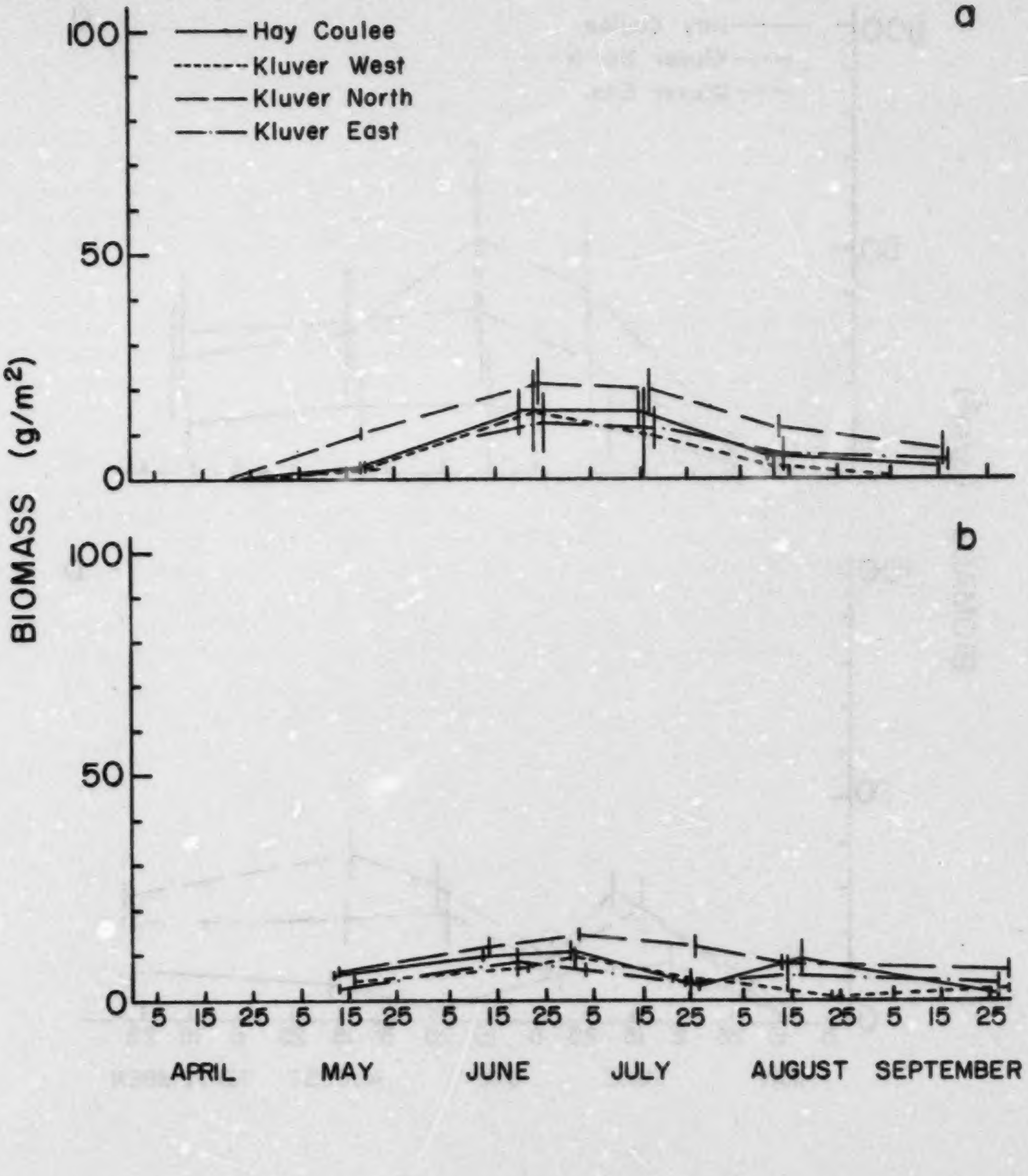


Figure 1.5. Production of cool season forbs at the Colstrip sites during the 1974 (b) and 1975 (a) growing seasons.

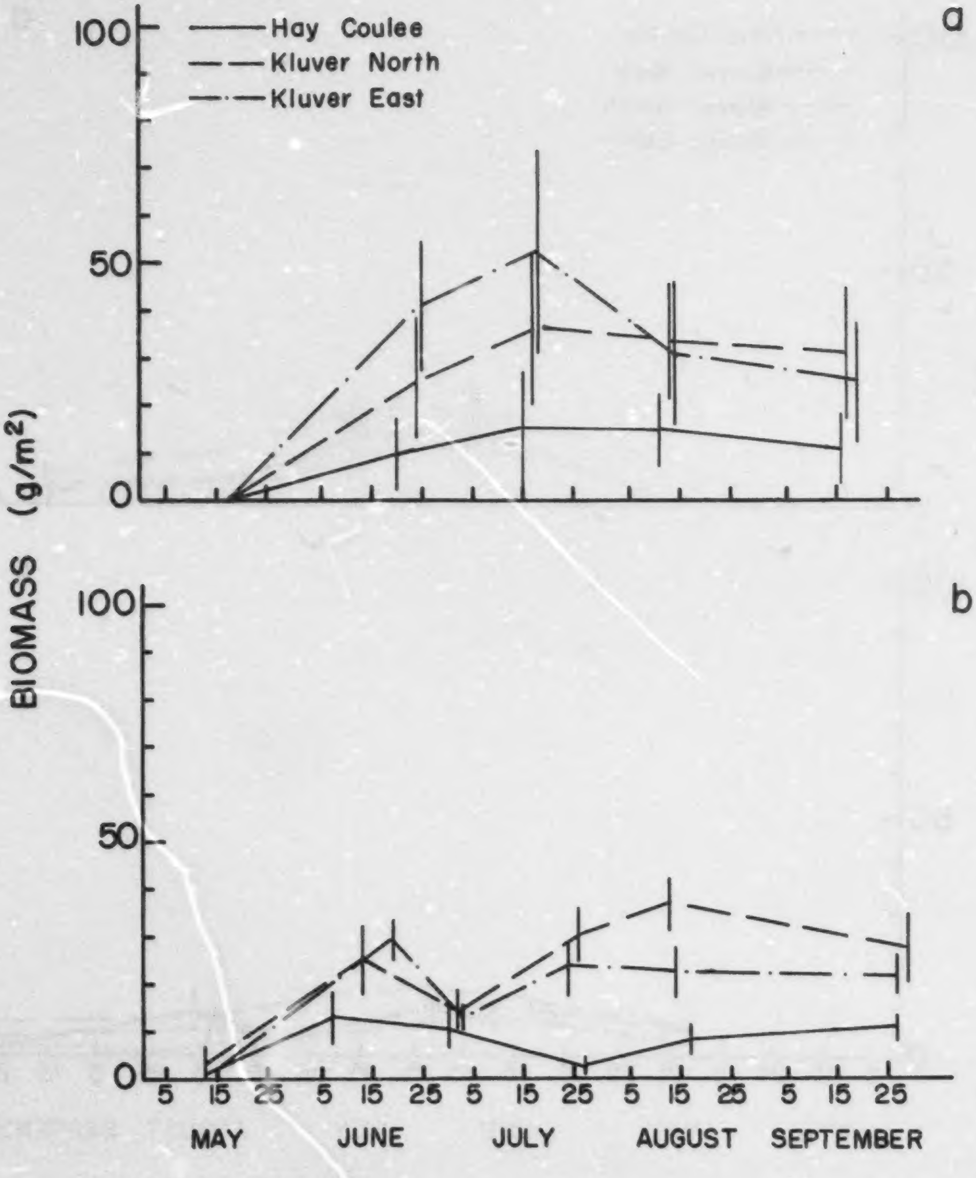


Figure 1.6. Production of half-shrubs at the Colstrip sites during the 1974 (b) and 1975 (a) growing seasons.

Warm season grasses are generally an unimportant component in terms of total productivity of these sites and very small differences among the two years were recorded (Figure 1.7). The Hay Coulee site had the greatest productivity of warm season grasses in both years and was approximately 15 $g/m^2$. Slight differences were seen for the remaining sites with an increase in warm season grasses for both the Kluver West and Kluver East sites.

Aboveground net primary production (ANPP) was calculated by summing the peak values of current live plus recent dead biomass for each functional group. Figure 1.8 shows ANPP for the four sites for 1974 and 1975 with the contribution of each functional group. In 1974 ANPP estimates ranged from 106 $g/m^2/yr$ for the Kluver East site to 123 $g/m^2/yr$ for the Kluver North site. The ANPP in 1975 was greater for all sites, ranging from 131 $g/m^2/yr$ for Kluver West to 165 $g/m^2/yr$ for Kluver East. The percentage contribution of the functional groups to ANPP was similar between years for all sites with the maximum differences on the order of 5% to 7%. An example of this is the data from the Kluver North site. Here the percentage contribution of half-shrubs increased 5% from 1974 to 1975, the same for cool season forbs decreased 7%, warm season grasses increased 5%, and cool season grasses decreased 4%. The explanation for these differences is related to the interaction of exclusion from grazing and increased precipitation in 1975. At this time we are not able to separate the effect of these influences.

## BELOWGROUND PRODUCER BIOMASS

The seasonal dynamics data for belowground biomass require further analyses and are not discussed in this report. Analyses of the depth distribution of root biomass are complete. Figure 1.9 presents the vertical distribution of root biomass for six sites over 2 years. Although there is some variability in the depth distribution of the root systems of these northern mixed prairie grasslands, on the average they are quite similar. Averaging over all sites in both years approximately 51% of the total root biomass is found in the 0-10 cm depth. This value decreases to 4% of the total root biomass found in the 50-60 cm depth. These averages are similar to root distributions reported by other researchers (Dodd et al., 1974; Bartos and Sims, 1974).

## LITTER DYNAMICS

Changes in litter standing crop on the Colstrip sites for 1974 and 1975 indicate general similarity between sites and years (Table 1.7). Although several dissimilarities among sites are apparent for the 1974 season and the early portion of the 1975 season, the variation between sites by the end of 1975 (August and September) was small. The early differences are attributed to lag effects from differential grazing histories prior to 1974; the authors conclude that, with respect to litter production and decomposition, the sites are now comparable and will likely remain so unless differences are induced by site-to-site variations in weather or air pollution.

## ARTHROPODS

The arthropod fauna of the four EPA field sites near Colstrip, Montana (Hay Coulee, Kluver West, Kluver North, and Kluver East) were sampled by three

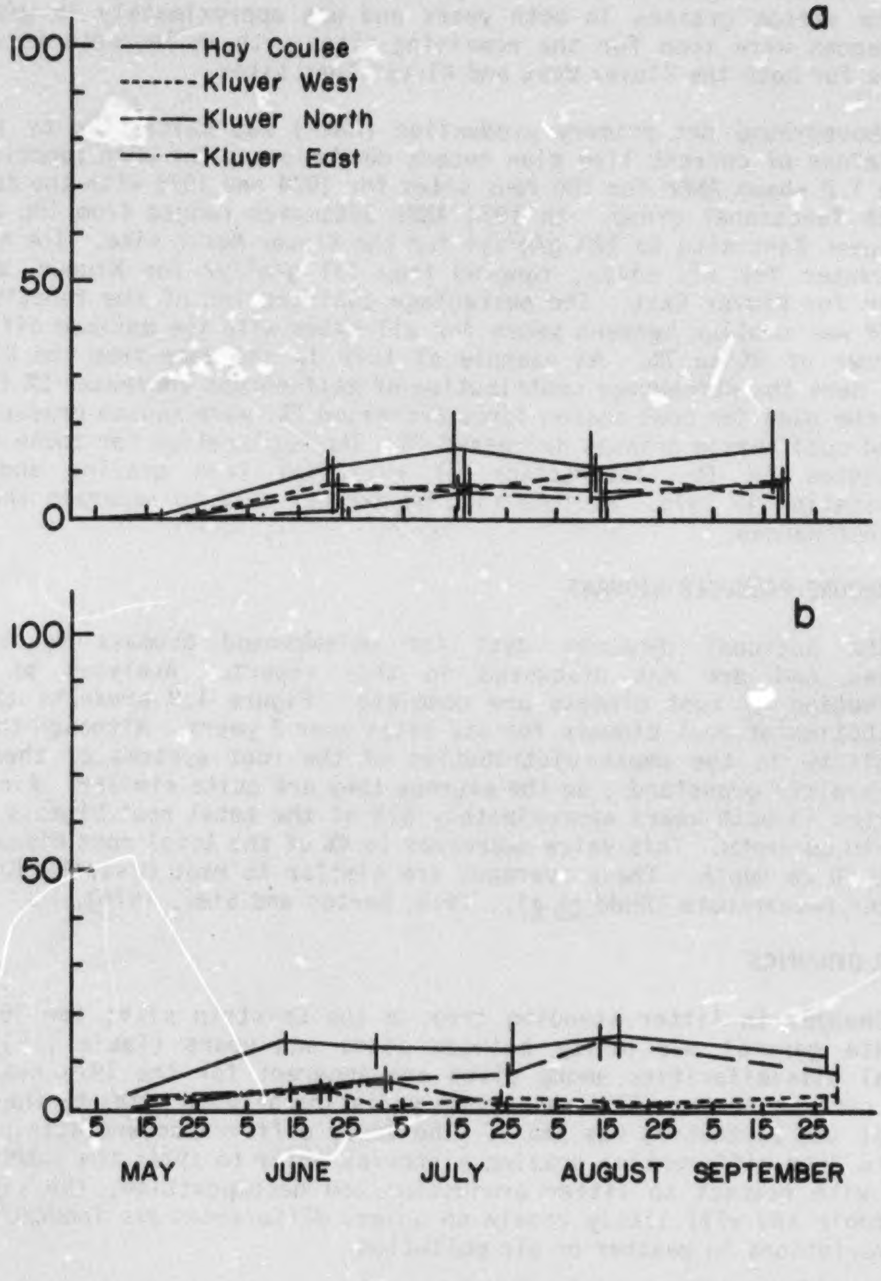


**Figure 1.7.** **Production of warm season grasses at the Colstrip sites during the 1974 (b) and 1975 (a) growing seasons.**

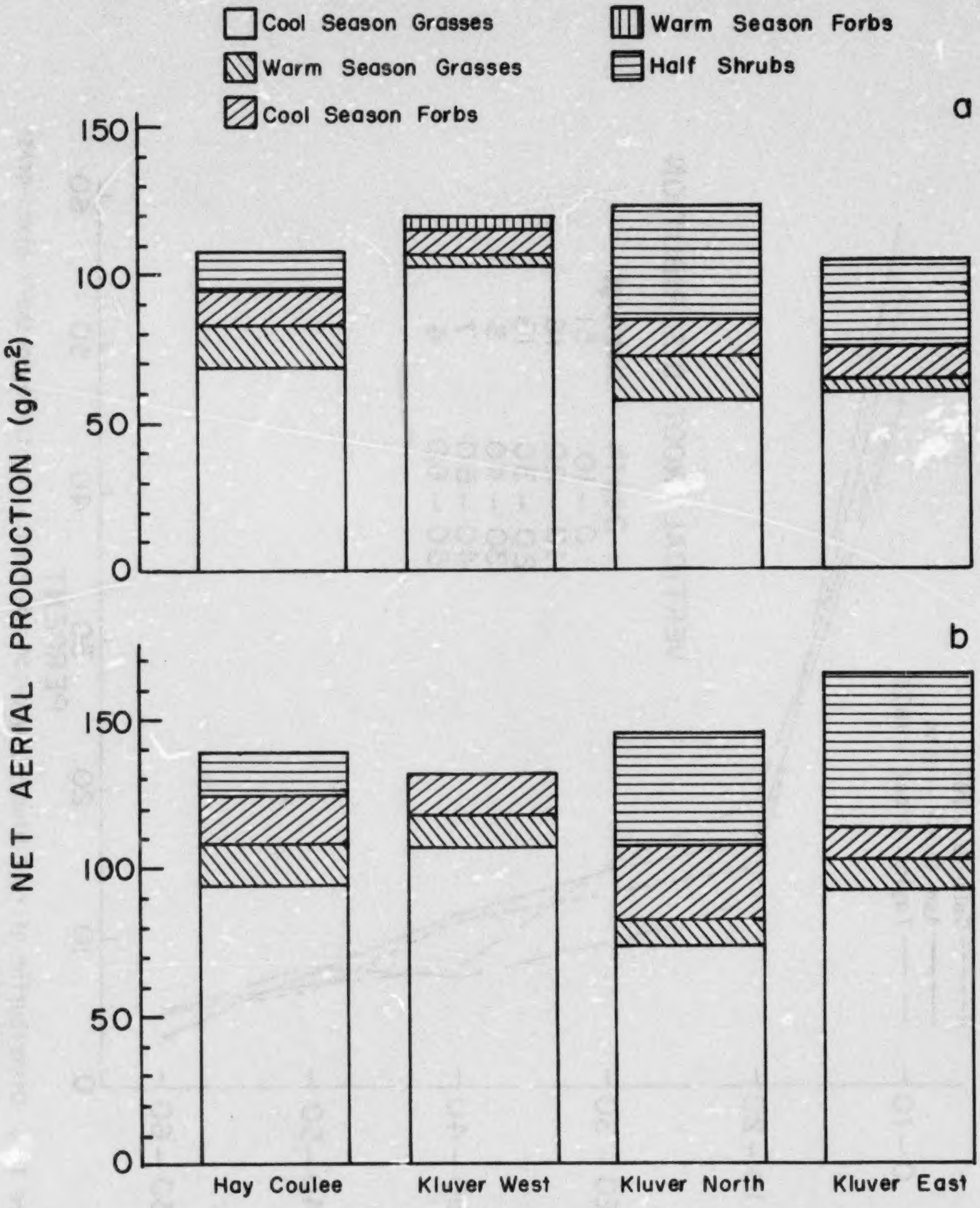
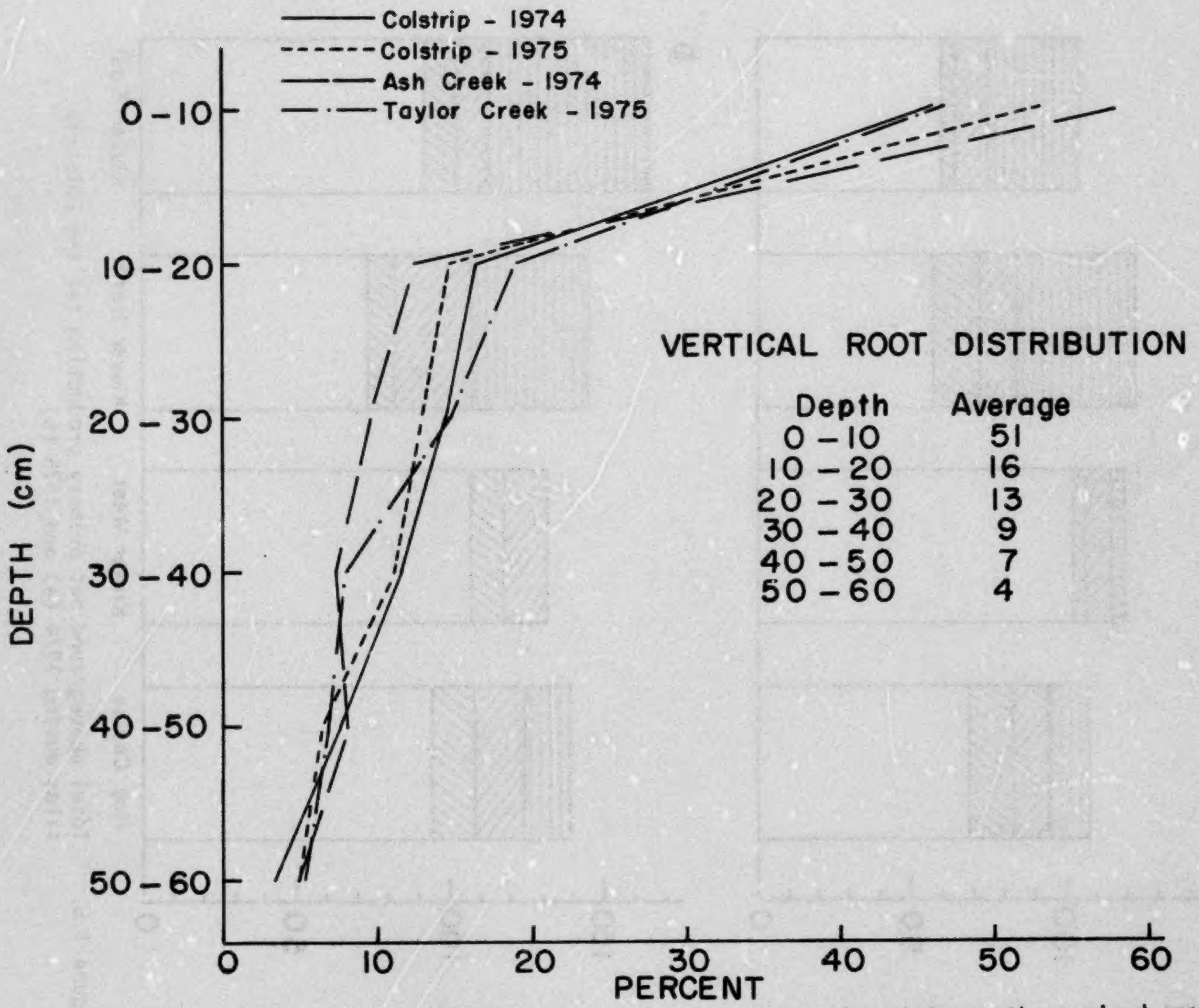


Figure 1.8. Total aboveground net primary production for the Colstrip sites during 1974 (a) and 1975 (b).



VERTICAL ROOT DISTRIBUTION

| Depth | Average |
|---|---|
| 0 – 10 | 51 |
| 10 – 20 | 16 |
| 20 – 30 | 13 |
| 30 – 40 | 9 |
| 40 – 50 | 7 |
| 50 – 60 | 4 |

Figure 1.9. Distribution of root biomass by depth increments for three northern mixed-grass prairie sites.

TABLE 1.7. LITTER STANDING CROP ($\bar{X}$ ± SE, ash free g/m$^2$) FOR COLSTRIP SITES, 1974 AND 1975.

| | Hay Coulee | | Kluver West | | Kluver North | | Kluver East | |
|---|---|---|---|---|---|---|---|---|
| Date | $\bar{X}$ | SE | $\bar{X}$ | SE | $\bar{X}$ | SE | $\bar{X}$ | SE |
| 1974 | | | | | | | | |
| 11 May | 161 | 17 | 177 | 13 | 234 | 21 | 158 | 16 |
| 11 June | 172 | 11 | 175 | 12 | 157 | 22 | 154 | 12 |
| 29 June | 201 | 13 | 183 | 6 | 169 | 15 | 171 | 19 |
| 26 July | 172 | 12 | 140 | 12 | 171 | 18 | 161 | 18 |
| 16 August | 128 | 12 | 139 | 19 | 198 | 10 | 153 | 18 |
| 26 September | 192 | 15 | 167 | 13 | 230 | 24 | 148 | 13 |
| $\bar{X}$ | 171 | | 163 | | 193 | | 157 | |
| 1975 | | | | | | | | |
| 22 April | 162 | 10 | | | 193 | 22 | 144 | 17 |
| 18 May | 170 | 14 | 159 | 9 | 205 | 19 | | |
| 20 June | 179 | 11 | 166 | 20 | 163 | 16 | 157 | 19 |
| 15 July | 154 | 15 | 160 | 10 | 218 | 32 | 152 | 14 |
| 11 August | 231 | 26 | 207 | 15 | 277 | 33 | 204 | 16 |
| 16 September | 218 | 18 | 191 | 14 | 189 | 16 | 206 | 39 |
| $\bar{X}$ | 186 | | 177 | | 207 | | 144 | |

different techniques, each designed for certain components of the total fauna. For the "aboveground arthropods," i.e., those occurring in the litter and standing vegetation, a quick trap system was used. An open-bottomed cage of known size was dropped over preselected, randomly located plots. The cage or quick trap was dropped from the end of an 18 ft boom mounted on a two-wheeled cart. The insects, vegetation, and surface litter were removed from the trap by a vacuum system and the arthropods extracted from the refuse by use of Berlese- or Tullgren-type extraction.

The "soil macroarthropods," i.e., those arthropods occurring below the surface litter and large enough to be sieved from the soil with a 1 mm opening sieve, were sampled by taking 12.5 cm diameter by 15 cm deep soil cores and wet sieving them. All material retained by the sieve was further separated by flotation in a saturated solution of magnesium sulfate ($MgSO_4$).

The "soil microarthropods," i.e., those occurring in the litter and soil and small enough to pass through a 1 mm opening sieve, were sampled by taking 5.0 cm diameter by 10.0 cm deep soil cores in such a way as to retain, as nearly as possible, the original soil structure. The arthropods were extracted by a Macfadyen high temperature gradient system. Leetham (1975) gives a detailed discussion of all the above techniques.

In the field at each randomly chosen sample point, one sample of each of the above types was taken with five such sample points being selected in each of two replicates of each treatment, i.e., field site. All arthropods collected were identified to family (genus and species where possible). Representatives of each group or category were dried at 65° C for 25 hr and weighed to obtain dry weight biomass data. There were five to six sample dates in each of the 1974 and 1975 seasons.

Because of some recently detected problems in the computer summarizations of the soil microarthropod data, a discussion of that portion of the arthropod fauna will be included in a later report.

Tables 1.8 and 1.9 give summarizations of the aboveground and soil macroarthropods by total counts (Table 1.8) and by the various trophic or functional groups (Table 1.9). From Table 1.8 it is apparent that generally there was a substantial difference between the two seasons with the 1975 season producing larger numbers of both aboveground arthropod biomass which shows a substantial reduction, possibly due to a shift in the dominant species between the two years. There appears to be considerable treatment or field site differences, however, the significance of these differences may be artificial due to the high variability of the data. Generally, the Kluver North (C) plot supported the highest arthropod populations in 1974 but that dominance changed in 1975. Since no statistical analyses have been made on the data, any significant treatment and/or year effects are unknown.

Table 1.9 gives a summary of the arthropod fauna by trophic group. Plant feeding types are all grouped together here rather than splitting them by their feeding types, i.e, chewing, sucking, pollen feeding, etc. The plant feeding and omnivorous types appear to be the dominant feeding types, both by numbers and biomass for both above and belowground arthropods. Within the treatments,

TABLE 1.8. SUMMARY, BY TOTAL COUNT, OF THE ARTHROPOD CENSUSING AT THE FOUR FIELD SITES NEAR COLSTRIP, MONTANA FOR THE 1974 AND 1975 SEASONS (A = Hay Coulee, B = Kluver West, C = Kluver North, D = Kluver East).

| | Numbers/m2[1/] | | | | Biomass g/m2[1/] | | | |
|---|---|---|---|---|---|---|---|---|
| Group | A | B | C | D | A | B | C | D |
| Aboveground arthropods | | | | | | | | |
| 1974 | 45.2 | 39.8 | 96.5 | 48.3 | .143 | .269 | .345 | .200 |
| 1975 | 136.1 | 99.3 | 122.2 | 109.2 | .119 | .174 | .126 | .082 |
| Soil macroarthropods | | | | | | | | |
| 1974 | 37.8 | 49.5 | 74.7 | 42.7 | .148 | .065 | .080 | .045 |
| 1975 | 82.2 | 148.3 | 62.5 | 69.5 | .574 | .551 | .620 | .356 |

[1/] Time weighted means.

TABLE 1.9. SUMMARIZATION BY TROPHIC OR FUNCTIONAL GROUP OF THE ARTHROPOD DATA GIVEN IN TABLE 1.8 (A = Hay Coulee, B = Kluver West, C = Kluver North, D = Kluver East).

| Group | Numbers/m$^2$[1] | | | | Biomass g/m$^2$[1] | | | |
|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | A | B | C | D |
| | | | | Unknown | | | | |
| Aboveground arthropods | | | | | | | | |
| 1974 | 2.9 | 1.4 | 2.2 | 5.8 | .001 | >.001 | .001 | .002 |
| 1975 | 11.5 | 15.7 | 20.2 | 18.4 | .002 | .001 | .001 | .001 |
| Soil macroarthropods | | | | | | | | |
| 1974 | 23.2 | 3.9 | 9.8 | 6.3 | .091 | .016 | .024 | .001 |
| 1975 | 30.1 | 75.2 | 3.5 | 19.6 | .072 | .013 | .004 | .018 |
| | | | | Plant Feeding | | | | |
| Aboveground arthropods | | | | | | | | |
| 1974 | 28.1 | 21.6 | 60.6 | 22.6 | .132 | .249 | .326 | .186 |
| 1975 | 49.0 | 49.1 | 63.0 | 50.4 | .085 | .150 | .100 | .065 |
| Soil macroarthropods | | | | | | | | |
| 1974 | 5.8 | 8.9 | 9.7 | 16.4 | .023 | .027 | .032 | .040 |
| 1975 | 20.9 | 18.8 | 25.1 | 24.8 | .332 | .112 | .410 | .263 |
| | | | | Predators | | | | |
| Aboveground arthropods | | | | | | | | |
| 1974 | 6.6 | 5.4 | 8.9 | 7.6 | .005 | .005 | .005 | .005 |
| 1975 | 17.1 | 8.9 | 13.7 | 15.3 | .008 | .005 | .012 | .003 |
| Soil macroarthropods | | | | | | | | |
| 1974 | 6.2 | 1.4 | 9.9 | -- | .023 | >.001 | .002 | -- |
| 1975 | 11.6 | 22.5 | 17.6 | 6.9 | .064 | .125 | .127 | .043 |
| | | | | Parasitoid | | | | |
| Aboveground arthropods | | | | | | | | |
| 1974 | 0.5 | 0.4 | 0.8 | 0.6 | >.001 | >.001 | .001 | .001 |
| 1975 | 1.0 | 0.6 | 1.4 | 0.4 | .001 | >.001 | .001 | >.001 |

(continued)

TABLE 1.9. (continued)

| Group | Numbers/m$^2$[1] | | | | Biomass g/m$^2$[1] | | | |
|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | A | B | C | D |
| Soil macroarthropods | | | | | | | | |
| 1974 | -- | 2.5 | 1.0 | -- | -- | .008 | .005 | -- |
| 1975 | -- | -- | -- | -- | -- | -- | -- | -- |
| | | | Omnivore | | | | | |
| Aboveground arthropods | | | | | | | | |
| 1974 | 5.0 | 5.0 | 20.0 | 7.2 | .001 | .001 | .004 | .002 |
| 1975 | 21.8 | 12.7 | 16.0 | 13.6 | .014 | .009 | .010 | .011 |
| Soil macroarthropods | | | | | | | | |
| 1974 | 3.0 | 25.9 | 23.7 | 7.0 | .002 | .007 | .005 | .001 |
| 1975 | 30.1 | 75.2 | 3.5 | 19.6 | .072 | .103 | .004 | .018 |
| | | | Scavenger | | | | | |
| Aboveground arthropods | | | | | | | | |
| 1974 | 2.1 | 5.9 | 3.3 | 4.3 | .003 | .013 | .005 | .004 |
| 1975 | 33.8 | 13.5 | 7.7 | 11.2 | .003 | .004 | .005 | .002 |
| Soil macroarthropods | | | | | | | | |
| 1974 | -- | 5.8 | 2.9 | 3.0 | -- | .004 | .003 | .003 |
| 1975 | 1.7 | 6.9 | 3.8 | 4.5 | .006 | .019 | .020 | .023 |
| | | | Non-feeding | | | | | |
| Aboveground arthropods | | | | | | | | |
| 1974 | -- | 0.1 | 0.1 | 0.1 | -- | >.001 | >.001 | >.001 |
| 1975 | 1.9 | 2.9 | 0.4 | -- | .006 | .009 | .001 | -- |
| Soil macroarthropods | | | | | | | | |
| 1974 | 1.8 | 2.4 | 16.8 | 11.4 | .010 | .004 | .009 | .006 |
| 1975 | 14.7 | 23.2 | 8.5 | 10.5 | .094 | .063 | .035 | .009 |

[1] Time weighted means.

Kluver North supports the highest numbers and biomass of plant feeders and the lowest number and biomass of scavengers. The switch in the relationship of numbers to biomass for aboveground plant feeders, i.e., lower biomass for higher numbers in 1975, appears to reflect a change in the dominant species types, as mentioned earlier. We hope a more in-depth analysis of the data will answer this question.

Since the data included here have not been statistically analyzed, the homogeneity of the four field plots is not clearly defined. This is due in part to the high variability of the data, a common characteristic of this kind of ecological data. When the statistical analyses are complete, a comparison of year and date information will provide a more detailed characterization of the field sites as examples of a northern mixed-grass prairie.

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## APPENDIX

## SOIL DESCRIPTIONS[1/]

Soil surveys were conducted at the Kluver East, Kluver West, Kluver North, and Hay Coulee sites. The morphological properties were examined to a depth of 152 cm in each of two soil excavation pits at each site. Samples were taken from all horizons in the profiles. Those samples are now being analyzed for physical and chemical properties.

Preliminary analyses based on morphological properties indicate that the soils at the Kluver East site belong to Kobar and Lonna series. At the Kluver West and Kluver North sites the Yamac series predominates, but much of the soil at the Kluver West site is very stoney and cobbly causing description to be difficult. The Hay Coulee site has soils of the Yamac and Lonna series. Representative profile descriptions from these series are presented in profiles 1-3.

Complete analyses and interpretation of these soils await completion of physical and chemical analyses. Physical properties being determined are texture, bulk density, and water-holding capacity. Chemical analyses will yield information for organic matter (carbon) pH, total N, total P, inorganic P, organic S, total S, inorganic S, cation exchange capacity, exchangeable bases (Ca, K, Mg, and Na), and lime.

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[1/] Soils were described by R. G. Woodmansee with the assistance of Lewis Daniels, Soil Survey Party Chief, USDA, SCS, Forsythe, Montana.

## PROFILE 1. Kobar Clay Loam

### Kluver East - North Pit

| Horizon | Depth (cm) | Description |
|---|---|---|
| A11 | 0-4 | Light brown gray (10 YR 6/2 dry) to dark brown (10 YR 4/3 moist); loam; moderate fine platy; hard when dry, friable when moist, slightly sticky and slightly plastic when wet; no lime present; abrupt, smooth boundary; many fine roots. |
| A12 | 4-12 | Light olive brown (2.4 YR 5/2 dry) to dark gray brown (10 YR 4/2 moist); clay loam; weak medium platy; hard when dry, friable when moist, sticky and plastic when wet; no lime present; clear, smooth boundary; many fine roots. |
| B2 | 12-18 | Pale brown (10 YR 6/3 dry) to dark brown (10 YR 4/3 moist); sandy clay loam; moderate medium prisms breaking to moderate medium blocks; hard when dry; friable when moist, sticky and plastic when wet; no lime present; clear, smooth boundary; many fine roots. |
| $B3_{ca}$ | 18-36 | Very pale brown (10 YR 7/3 dry) to dark brown (10 YR 5/4 moist); sandy clay loam; weak coarse prisms breaking to moderate medium and fine blocks; hard when dry, friable when moist, sticky and plastic when wet; lime present; gradual, smooth boundary; roots common. |
| $C1_{ca}$ | 36-120 | Very pale brown (10 YR 7/3 dry) to yellowish brown (10 YR 5/4 moist); sandy clay loam; massive; hard when dry, friable when moist, sticky and plastic when wet; lime present; gradual smooth boundary; roots common to 45 cm and few roots to 120 cm. |
| $C2_{ca}$ | 120-152 | Very pale yellow (10 YR 7/3 dry) to yellowish brown (10 YR 5/4 moist); clay loam; massive; hard when dry, friable when moist, sticky and plastic when wet; lime present; few roots present. |

**PROFILE 2.** Lonna Loam

Hay Coulee - North Pit

| Horizon | Depth (cm) | Description |
|---|---|---|
| A1 | 0-12 | Light brown gray (2.5 YR 6/2 dry) to dark brown crushed (10 YR 4/3 moist) and dark gray brown coated (10 YR 4/3 moist); loam; weak medium platy; slightly hard when dry, friable when moist, slightly sticky, slightly plastic when wet; no lime; clear smooth boundary; many fine roots. |
| $B2_{ca}$ | 12-22 | Pale brown (10 YR 6/3 dry) to yellowish brown crushed (10 YR 5/4 moist) and dark brown coated (10 YR 4/3 moist); silty clay loam; weak medium prisms breaking to moderate medium and fine blocks; hard when dry, friable when moist, sticky and plastic when wet; lime present; gradual smooth boundary; many fine roots. |
| $C1_{ca}$ | 22-60 | Very pale brown (10 YR 7/3 dry) to yellowish brown (10 YR 5/4 dry); silty clay loam; weak, very coarse platy breaking to moderate fine blocks (plates not of pedogenic origin); hard when dry, firm when moist, sticky and plastic when wet; lime present; gradual smooth boundary; many fine roots to 30 cm, common fine roots 30-60 cm. |
| $C2_{ca}$ | 60-98 | Very pale brown (10 YR 7/3 dry) to yellowish brown (10 YR 5/4 moist); silty clay loam; massive; hard when dry, friable when moist, sticky and plastic when wet; lime present; gradual smooth boundary; common fine roots to 80 cm, few fine roots to 98 cm. |
| $C3_{ca}$ | 98-152 | Very pale yellow (10 YR 7/3 dry) to light olive brown (2.5 YR 5/4 moist); silty; massive; slightly hard when dry, friable when moist; lime present; few roots present. |

PROFILE 3. Yamac Silt Loam

Kluver West - West Pit

| Horizon | Depth (cm) | Description |
|---|---|---|
| A1 | 0-10 | Brown (10 YR 5/3 dry) to dark gray brown (10 YR 4/2 moist); fine sandy loam; soft medium platy; soft when dry, very friable when moist, nonsticky, slightly plastic when wet; no lime; clear, smooth boundary; many fine roots. |
| B2 | 10-31 | Yellowish brown (10 YR 5/4 dry) to brown to dark brown crushed (10 YR 4/3 moist), dry gray brown coated (10 YR 4/2 moist); sandy clay loam; moderate medium prisms breaking to moderate medium blocks; hard when dry, friable when moist, sticky and plastic when wet; no lime; clear, smooth boundary; fine roots common. |
| $B3_{ca}$ | 31-40 | Pale brown (10 YR 6/3 dry) to dark gray brown (2.5 YR 4/2 moist); sandy clay loam; weak coarse prisms breaking to weak coarse and medium blocks; hard dry, friable when moist, sticky and plastic when wet; lime present but not visible; gradual smooth boundary; roots common. |
| $C1_{ca}$ | 40-118 | Very pale brown (10 YR 7/3 dry), too cobbly and mixed to determine moist color; cobbly (upper 7 cm); sandy clay loam; massive; hard when dry, friable when moist, sticky and plastic when moist; small threads and masses of lime; gradual and wavy boundary; roots common 40-50 cm, few roots 50-118 cm. |
| $C2_{ca}$ | 118-156 | Light gray (2.5 YR 7/2 dry), too variable for moist color; cobbly sandy loam; massive; too cobbly for consistence; lime coatings on cobbles; few roots. |

Lower horizons of this profile contain unweathered fragments similar to nearby uplands. Most Yamac soils of EPA study sites do not contain these cobble layers. The east end of Kluver West contains so much cobble and gravel that the pit was not described.

## SECTION 2

# PLANT COMMUNITY STUDIES IN THE VICINITY OF COLSTRIP, MONTANA

by

J. E. Taylor and W. C. Leininger

## INTRODUCTION

The plant community monitoring aspects of the Colstrip Coal-fired Power Plant Project are generally divided into studies of more-or-less quantitative plant population and community attributes (diversity, phenology, canopy coverage, productivity) and photographic surveillance, both from ground and aerial vantage points. This section will deal with diversity, canopy coverage, and phenology studies.

The procedures and data reported here are interrelated with concurrent studies of bird and mammal populations (including diversity), plant pathology, and lichen ecology. In total, all of these studies are contributing to the development of a generalized, comprehensive approach to air pollution effects monitoring in biological systems.

The objectives of this aspect of the project are to:

1. Document pre-treatment native plant communities in areas likely to be affected by the power plants under investigation and on areas to be stressed artifically with pollutants.
2. Develop measurement techniques and monitor changes in plant community structure, diversity, phenology, and speciation following air pollution stress.
3. Develop detailed vegetation maps of the study areas.
4. Provide data for simulation models to predict bioenvironmental changes resulting from fossil fuel power generation in other areas.

The work was initiated on July 15, 1974. Research from previous field seasons has been reported by Taylor *et al*. (1975 and 1976). The present report is based on the data collected during the 1975 field season.

This discussion is divided into two principal parts: diversity studies, using standard indices derived from information theory, and phenology studies, using an index system of the authors' design.

The underlying hypothesis of this work is that, as a plant community is stressed with the application of any external perturbation, including air pollution, and physiological changes in members of the community which result in changed rates of vital functions should be shown by changed species composition and/or rates of seasonal growth processes. If species composition is affected, with either loss of sensitive species, or re-ordering of species dominance in the community, this should be reflected in the species diversity observed. If rates of normal growth and reproductive functions are influenced, the phenological profiles of component species should change. In the case of the Zonal Air Pollution System (ZAPS) plots, where sulfur dioxide is applied at controlled rates to native vegetation, both of these indications have been observed (see Section 11).

Both responses to pollution could be studied with biomass/productivity data, such as those collected by Dodd and associates (see Sections 1 and 10). However, the authors are interested in developing a non-destructive methodology which might be used where biological material was in limited supply, or where complementary observations could be made, serving to fortify biomass data by mutual corroboration.

## SPECIES DIVERSITY STUDIES

In 1975, 3 sampling times were used, representing (a) "peak of green" (June 10-11), (b) summer growth and maturity and warm season "peak of green" (July 10-12), and (c) late season maturity (September 16-17).

Plots within study areas were located by placing a cord with meter-spaced knots in a random meandering pattern through the sample area. The 2 x 5 dm plot frames were placed at each knot and canopy coverage estimated using the procedure of Daubenmire (1959). At the same time, species were counted, the numerical data to go into diversity indices. Previous experimentation had indicated that 2 sets of 20 frames per plot constituted a statistically adequate sample (i.e., additional observations did not contribute to increased sensitivity of diversity indices).

The diversity index used was the Shannon-Weaver function (Shannon and Weaver 1949):

$$H' = - \sum_{i=1}^{S} \frac{Ni}{N} \ln \frac{Ni}{N}$$

Where H' = Index of diversity
S = number of species
Ni = number of ith species
N = total number of all species

The index was calculated in two different ways. In using number data (plant density), the index was calculated for each of the 50 individual frames, and the mean of these values was tabulated and graphed. With canopy coverage data, the index was calculated as an accumulated figure over the entire set of 40 frames.

The first approach allows statistical comparisons among data sets; the second was used to evaluate the feasibility of applying indices to canopy coverage data.

Further, the number-based indices were calculated with a modification of the Shannon-Weaver function which since has been discontinued. Thus, direct comparisons between indices are not legitimate, but relative tendencies should be meaningful.

## SPECIES DIVERSITY (CANOPY COVER) FOR THE COLSTRIP STUDY SITES

Diversity indices were calculated using coverage data from all sites and dates of sampling.

Canopy cover diversity for the Colstrip locations are presented in Figures 2.1, 2.2, and 2.3, showing data for 1974, 1975, and 1976, respectively. There are definite diversity differences among sites, and they are quite consistent over years. Magnitudes change, but relative diversity is reasonably constant.

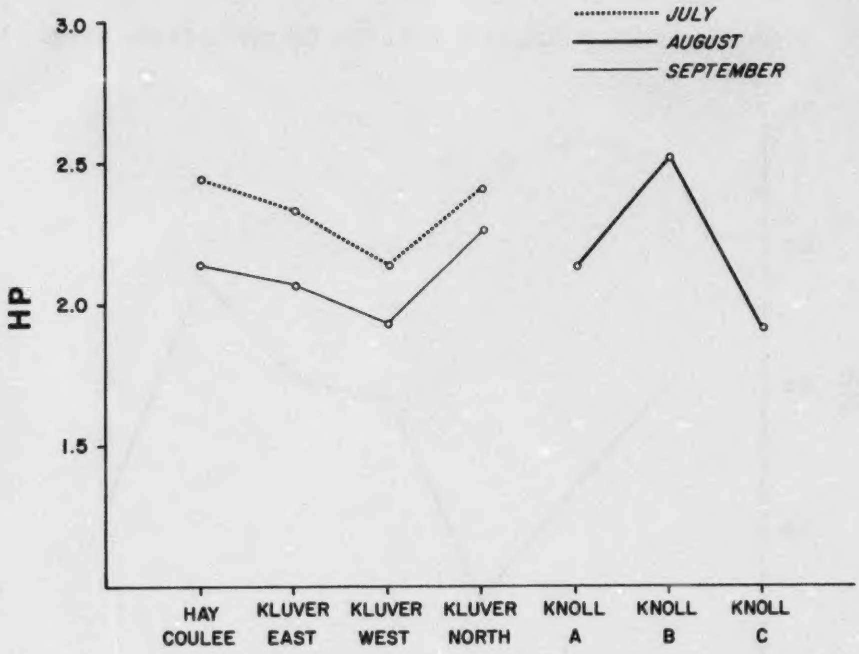

Figure 2.1. Shannon-Weaver function (HP) for Colstrip Study sites, 1974.

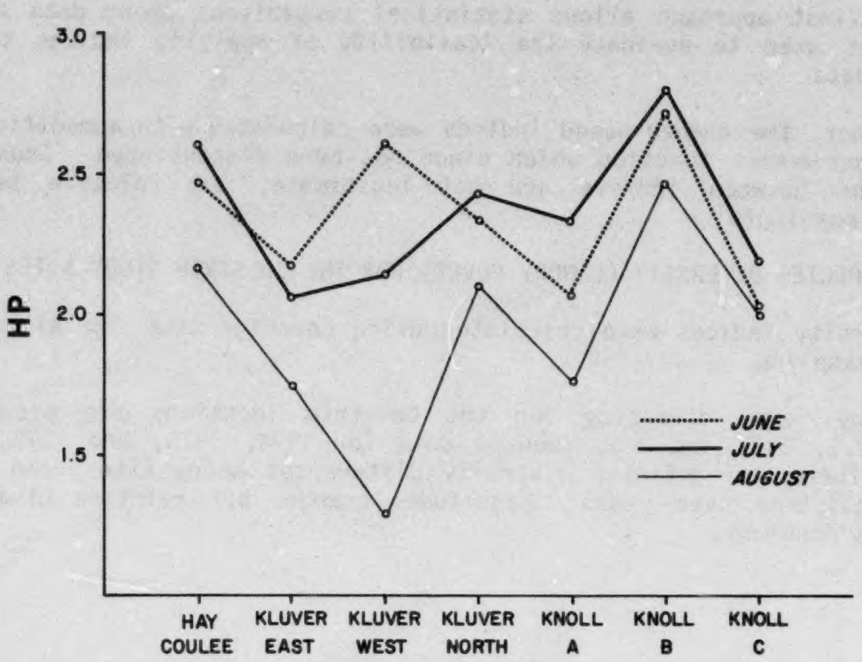

Figure 2.2. Shannon-Weaver function (HP) for Colstrip Study sites, 1975.

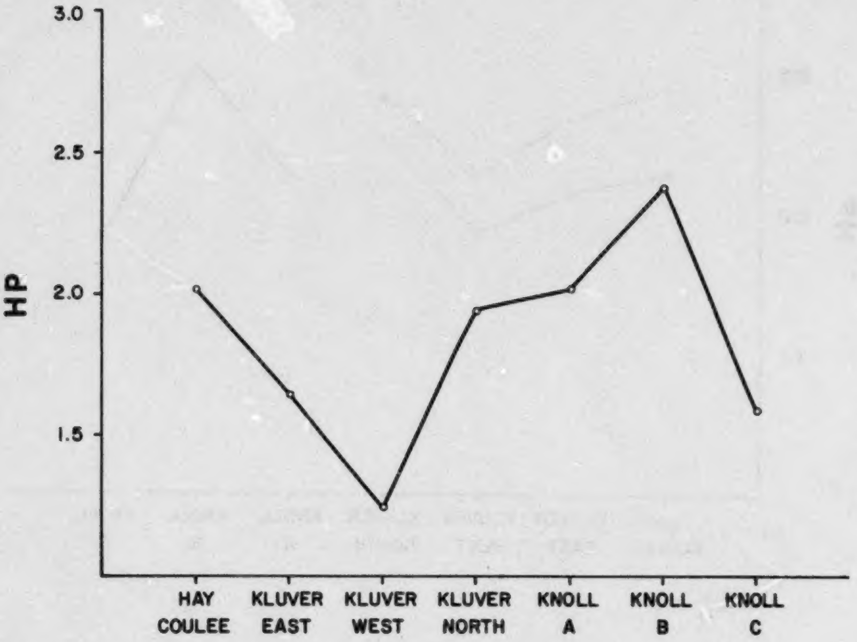

Figure 2.3. Shannon-Weaver function (HP) for Colstrip Study site, August, 1976.

These site differences can be related to plant species dominance and density. For example, Kluver West has a low diversity due to the strong dominance of needle-and-thread grass (Stipa comata) and the paucity of other species. Soil and microclimate variation, as well as grazing history, are the likely causes of this situation. Thus, comparisons among sites probably are of limited value.

The McRae Knolls, which are adjacent sites of similar grazing history and climate show diversity differences because of edaphic factors among sites. This is reflected primarily in species composition. When analyzed for range condition, Knolls A and C were in good condition and Knoll B was excellent. This estimate was based on percent of climax vegetation for the sites. The fact that the diversity indices show a similar pattern suggests a relationship between range condition and diversity, which is consistent with the results of many other studies.

Seasonal differences in diversity follow previously reported trends (Taylor et al., 1976). As the season progresses, many of the ephemeral forbs and cool-season grasses disappear or diminish in their contributions to total canopy coverage.

## PHENOLOGY STUDIES

Phenology data has been recorded since 1974 to establish a baseline data set.

The authors hypothesize that phenology will be a less sensitive approach than diversity in detecting pollution effects. This is primarily because of the large sample size required and the need to accumulate observations over a number of years in order to discriminate between "normal" and "abnormal" growth patterns. Therefore, the authors are continuing the data collection, and in fact are increasing the frequency of sampling, especially in the spring and early summer.

### PROCEDURES

A phenologic scorecard designed by the authors was used (Taylor et al., 1975); the categories are shown in Table 2.1. This classification has been modified since last season, with the insertion of the early flowering stage. With this addition, the system seems to fit a number of vegetation types and has generated considerable interest from various fields of ecological research. On each visit to an experimental site, a reconnaissance survey is made. Each recognizable plant species is characterized as to its modal phenologic stage. The scorecard approach attempts to quantify significant growth stages without resorting to counts or measurements.

The data are stored for further comparison and future analyses.

TABLE 2.1. PHENOLOGY CODES.

| Code | Stages |
|---|---|
| 1 | Cotyledon (newly germinated) |
| 2 | Seedling |
| 3 | Basal Rosette |
| 4 | Early greenup, veg. buds swelling |
| 5 | Vegetative growth, twig elongation |
| 6 | Boot stage, flower buds appearing |
| 7 | Shooting seed stalk, floral buds opening |
| 8 | Early flowering |
| 9 | Flowering, anthesis |
| 10 | Late flowering |
| 11 | Fruit formed |
| 12 | Seed shatter, dehiscence |
| 13 | Vegetative maturity, summer dormancy, leaf drop |
| 14 | Fall greenup |
| 15 | Winter dormancy |
| 16 | Dead |

## DISCUSSION

For purposes of elucidation, the index values (stages) for Kluver North are displayed graphically in Figures 2.4 (grasses) and 2.5 (forbs). The differences among species curves for grasses are clearly related to longevity and typical growth patterns (cool- vs. warm-season species).

Forbs and shrubs show more interspecific variation in phenology than do grasses, but site differences are inconsistent. This probably is due to the unusually favorable growing conditions during summer, 1975, following the later spring. The authors recognize that since the stages comprise an unscaled, arbitrary series, the resulting graphs should be viewed only as generalized profiles of plant growth and not subjected to quantitative analysis.

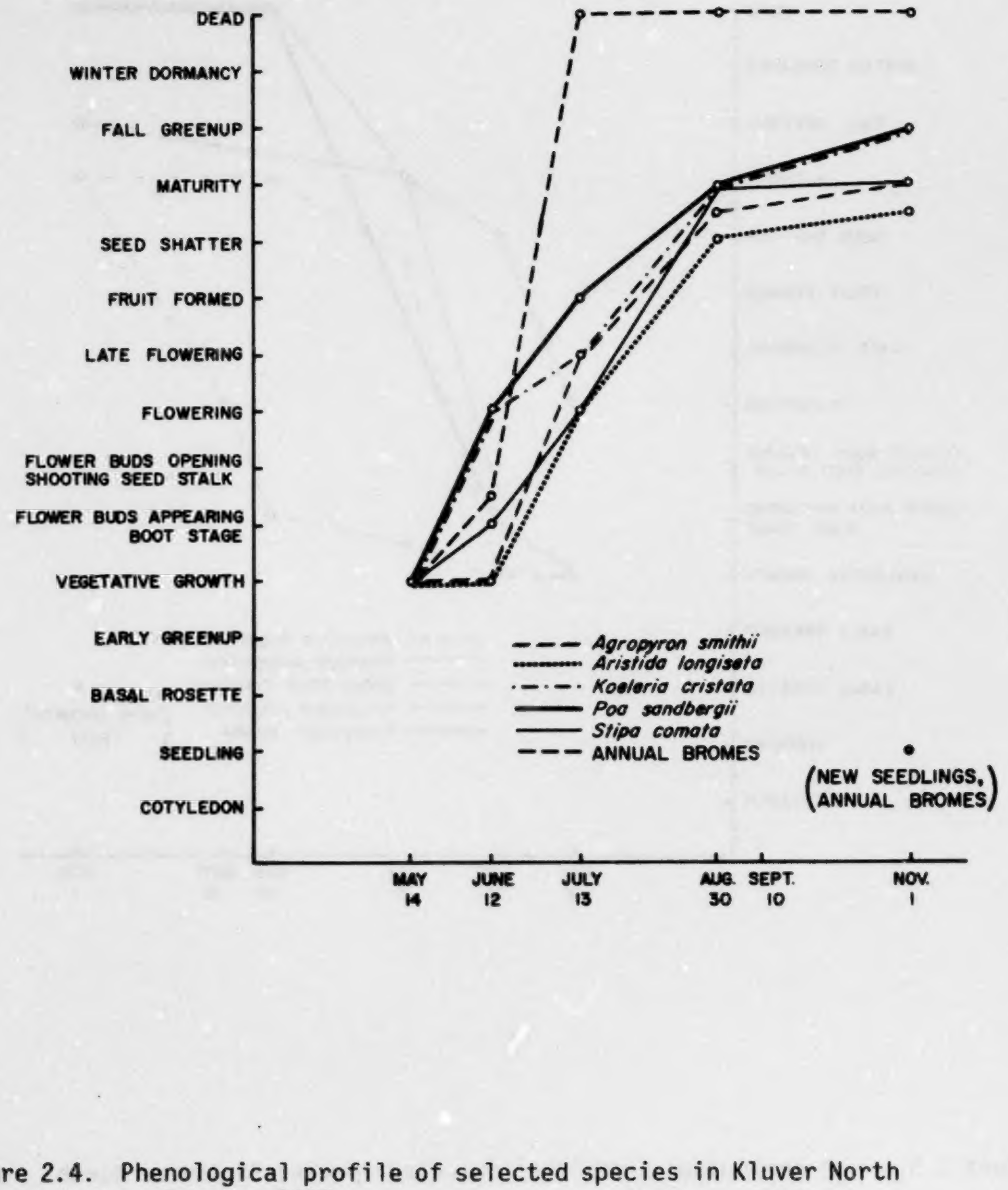

Figure 2.4. Phenological profile of selected species in Kluver North exclosure, 1975.

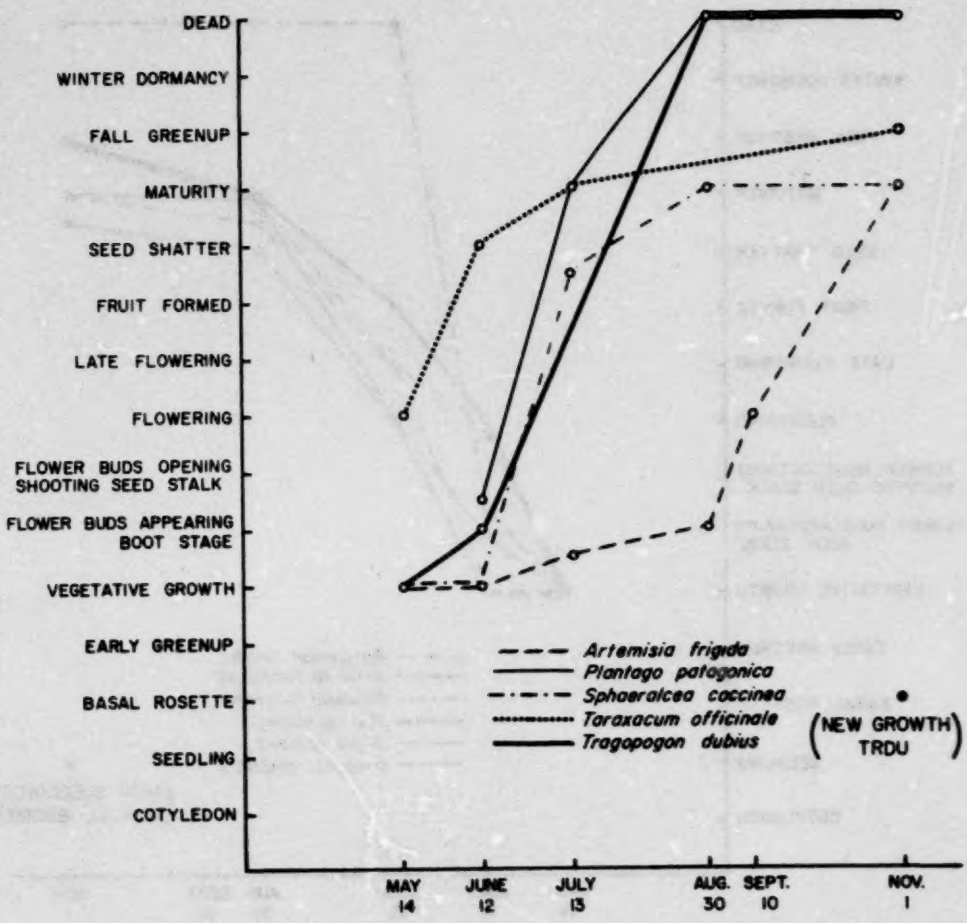

Figure 2.5. Phenological profile of selected species in Kluver North exclosure, 1975.

The development of analytical procedures whereby quantitative comparisons can be made among species, locations, seasons, and treatments is a continuing part of this research.

## PLANT COLLECTIONS

An ongoing program of plant collection has been under way since the initiation of the field phases of the project. Voucher species are deposited in the Herbarium of Montana State University. In addition, field specimens are maintained for reference of crews and others for consistency of naming, correctness of identification, elimination of duplicate numbers in Unknowns, etc.

Small field specimens are taken in plastic covers, following the procedure of Burleson (1975).

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# SECTION 3

# SOIL AND EPIPHYTIC LICHEN COMMUNITIES OF THE COLSTRIP, MONTANA AREA

by

S. Eversman

## INTRODUCTION

Numerous field studies have used lichen distribution patterns to locate areas of air pollution. The studies show that fewer lichen species occur in polluted areas (LeBlanc and Rao, 1975), and that lichen species transplanted into polluted areas lose vigor and/or die (LeBlanc and Rao, 1975). The Colstrip project in southeast Montana provides the rare opportunity to map lichen communities and obtain baseline physiological and anatomical information before the two 350 megawatt coal-burning power plants begin operation. This project will be able to establish the time progression of low-level pollution effects on a previously pristine area.

The specific objectives are to:

1) Determine baseline lichen community information for the grassland and ponderosa pine vegetation types.

2) Establish the baseline anatomical and physiological condition of two native lichen species.

3) Continually monitor these characteristics to detect changes caused by increasing air pollution.

4) Compare lichen monitoring results with measurements taken simultaneously on associated vascular vegetation by other investigators.

## LOCATIONS AND METHODS

The lichen study sites in the Colstrip area are shown in Figure 3.1. Table 3.1 provides this information for each site.

Samples of all observed lichen and moss species on soil and rocks have been collected from sites G1-G7 (grassland sites 8-97 km from the Colstrip power plants). Specimens were taken outside the exclosure fences at the common CFPP project research sites at Hay Coulee, Kluver North, Kluver West, Kluver East, (G1 + G3-G5 on Figure 3.1).

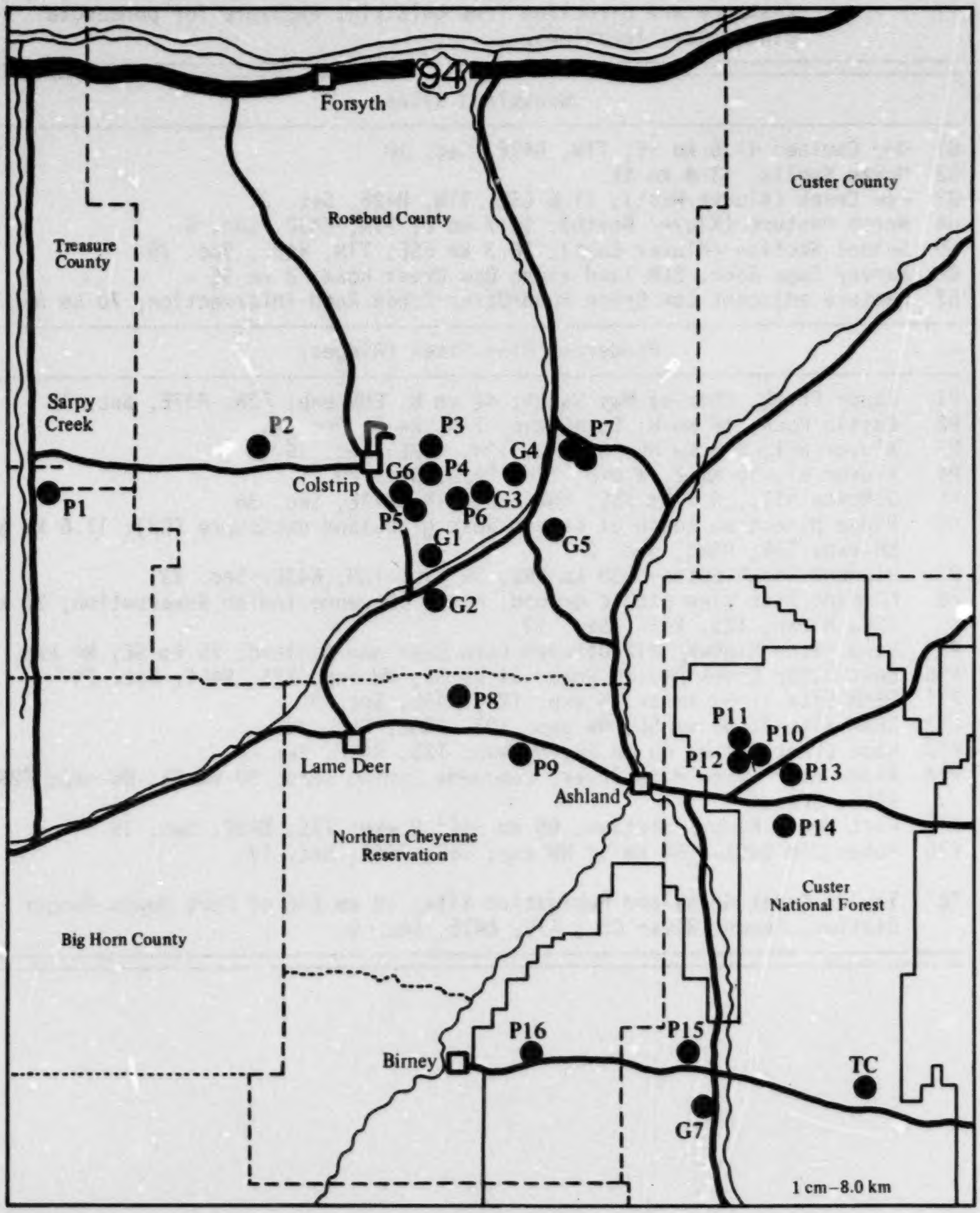
94
Forsyth
Custer County
Rosebud County
Treasure County
Sarpy Creek
P2
P3
P7
G4
P4
G6
Colstrip
P1
P5
P6
G3
G5
G1
G2
P8
P11
P10
P12
P13
Lame Deer
P9
Ashland
P14
Northern Cheyenne Reservation
Custer National Forest
Big Horn County
P16
P15
Birney
TC
G7
1 cm–8.0 km

Figure 3.1. Location of Field Study Sites in the Colstrip area. P1-P16 are ponderosa pine sites on ridges; G1-G7 are grassland sites.

TABLE 3.1. DESCRIPTION OF LICHEN COLLECTION SITES, COLSTRIP AREA (Name; air distance and direction from Colstrip; exposure for ponderosa pine sites; location).

| | |
|---|---|
| | Grassland Sites |
| G1 | Hay Coulee; 11.6 km SE; T1N, R42E, Sec. 28 |
| G2 | McRae Knolls; 13.8 km SE |
| G3 | Cow Creek (Kluver West); 11.6 ESE; T1N, R42E, Sec. 2 |
| G4 | North Pasture (Kluver North); 14.7 km E; T1N, R43E, Sec. 6 |
| G5 | School Section (Kluver East); 18.3 km ESE; T1N, R43E, Sec. 15 |
| G6 | Harvey Sage Site, BLM land along Cow Creek Road; 8 km SE |
| G7 | Pasture adjacent Cow Creek Road-Otter Creek Road intersection; 70 km SSE |
| | Ponderosa Pine Sites (Ridges) |
| P1 | Sarpy Creek, Charles May Ranch; 44 km W; ENE exp; T2N, R37E, Sec. 36 |
| P2 | Castle Rock; 16 km W; E, NE exp; T1N, R41E, Sec. 36 |
| P3 | Kluver NE1; 9.6 km NE; SW exp; T2N, R42E, Sec. 16 |
| P4 | Kluver E1; 10 km E; W exp; T2N, R42E, Sec. 29 |
| P5 | D.McRae hill; 9.6 km SSE; NNW exp; T1N, R42E, Sec. 36 |
| P6 | Ridge direction south of Kluver West grassland exclosure (G3); 11.6 km S; NW exp; T1N, R42E, Sec. 2 |
| P7 | Diamond Ranch Buttes; 28 km ENE; SW exp; T2N, R43E, Sec. 22 |
| P8 | Morning Star View picnic ground, North Cheyenne Indian Reservation; 26 km SSE; N exp; T2S, R41E, Sec. 12 |
| P9 | Stop along Highway 212 between Lame Deer and Ashland; 35 km SE; NE exp. |
| P10 | East Otter Creek Divide Road; 54 km SE; NW exp; T2S, R46E, Sec. 24 |
| P11 | SEAM Site 1; 52 km SE; W exp; T2S, R46E, Sec. 22 |
| P12 | SEAM Site 2; 50 km SE; NW exp; T2S, R46E, Sec. 22 |
| P13 | Home Creek Butte; 60 km SE; NW exp; T2S, R46E, Sec. 4 |
| P14 | Ridge near Three Mile Creek, Lemonade Spring Road; 90 km SE; NW exp; T2S, R46E, Sec. 4 |
| P15 | Fort Howes Ranger Station; 65 km SSE; N exp; T6S, R45E, Sec. 19 |
| P16 | Poker Jim Butte; 64 km S; NW exp; T6S, R44E, Sec. 17 |
| TC | Taylor Creek Grassland Fumigation Site; 18 km ESE of Fort Howes Ranger Station, Powder River Co.; T7S, R47E, Sec. 9 |

Ten fenceposts, serving as permanent observation points, were placed inside each of the exclosures where grass cover was the lowest and lichen cover was the greatest. The point-drop method (Hanson, 1950) was used to record ground cover (bare soil, grass, litter, moss, forb, lichen species) at each fencepost. The center of the point-drop frame was placed on the north side of each fencepost and 200 points per exclosure were recorded.

Sixteen ponderosa pine study sites (P1-P16) are located on ridges 8-97 km from Colstrip; 13 of them are exposed ridges that face toward the power plant stacks. Ten sites (P1-P5, P8, P11-P14, P16) have sulfation plates placed there by other researchers (Gordon, 1975; Gordon *et al.*, 1976). Site P10 was used as a control and source of transplant material; P9 was used as a transplant source in 1975 only. Samples of lichens and mosses from all substrates have been collected from all sites.

Ten trees on each site (excluding P9) are permanently marked for annual determination of percentage of cover and frequency of each lichen species present. Cover classes 1 - 5 (1 = 1 - 5% cover; 2 = 6 - 25%; 3 = 26 - 50%; 4 = 51 - 75%; 5 = 76 - 100%) are recorded for each species encountered on each of the four compass sides of each tree (north = northeast to northwest; east = northeast to southeast; etc.). Midpoints of each cover class are used for calculations (Tables 3.3 and 3.4).

Transplants to some sites were made by wiring ponderosa pine branches covered with *Usnea hirta* onto selected branches of 10 pine trees facing toward the power plant. Site P10 was the source of all transplant material in 1975 and 1976. Transplants were made to sites P1-P6 to augment local *U. hirta* populations available for collection and to place sampling specimens in locations more exposed than those of existing populations. Transplants were made to sites P8, P15, and P16 to provide comparisons with the native populations to detect effects of transplanting. Site P7 had no epiphytic lichens at all.

*Parmelia chlorochroa* or *Usnea hirta* samples are collected periodically from test sites (except P9) for manometric determination of respiration rates, analysis of total sulfur (as $SO_4$) content by the Montana State University Soils Testing Laboratory (Eversman, 1975), and for microscopic examination of algal color and plasmolysis.

Plasmolysis percentage is determined by preparing three slides of three different plants from each site, counting 100 algal cells on each slide, and recording the number of plasmolyzed cells out of each 100 counted.

## RESULTS

Table 3.2 lists the primary lichen species on seven grassland sites. *Cladonia* spp. squamules are the dominant lichen types on the soil at all exclosure sites; species identification has not been made because the reproductive structures are very rarely found. Therefore, the other most abundant species, *Parmelia chlorochroa*, is used for analytical work.

TABLE 3.2. GROUND COVER OF THE PRIMARY LICHEN SPECIES ON 7 GRASSLAND SITES. PERCENTAGES DETERMINED BY THE POINT-DROP METHOD. + = present, less than 0.5% x = present in undetermined quantities.

| Lichen | Hay Coulee G1 | McRae Knolls G2 | Kluver West G3 | Kluver North G4 | Kluver East G5 | Harvey G6 | Pasture G7 |
|---|---|---|---|---|---|---|---|
| *Cladonia* spp. | 23.3% | x | 27.0 | 24.0 | 13.6 | x | x |
| *Parmelia chlorochroa* | 16.7 | x | 16.5 | 9.5 | 10.0 | x | x |
| *Collema tenax* | 5.0 | x | 2.5 | 2.0 | 2.7 | x | x |
| *Fulgensia fulgens* | + | x | 0.5 | 1.0 | + | x | x |
| *Buellia epigaea* | + | x | + | + | + | x | |
| *Dermatocarpon lachneum* | + | | + | + | + | x | |
| *Squamarina lentigera* | + | x | | | | x | |
| *Lecidea decipiens* | + | | | | | x | |
| *Acarospora schleicheri* | + | | | | | | |
| *Toninia coeruleonigricans* | + | | | | | | |
| *Agrestia hispida* | + | | | | + | x | |

TABLE 3.3. PONDEROSA PINE SITES: PERCENTAGE OF TOTAL LICHEN COVER, FREQUENCY (Number of Trees Having Lichens), NUMBER OF EPIPHYTIC LICHEN SPECIES AT THAT SITE.

| Site | % cover | Frequency | Number of species |
|---|---|---|---|
| P10, East Otter Creek | 39.75 | 10 | 1 |
| P12, SEAM Site 2 | 21.00 | 10 | 5 |
| P14, 3-Mile | 15.25 | 10 | 4 |
| P11, SEAM Site 1 | 13.63 | 10 | 6 |
| P16, Poker Jim | 12.88 | 10 | 6 |
| P13, Home Creek Butte | 11.75 | 10 | 7 |
| P15, Fort Howes | 10.81 | 10 | 7 |
| P5, D. McRae | 9.13 | 8 | 8 |
| P3, Kluver NE1 | 6.88 | 8 | 7 |
| P2, Castle Rock | 6.38 | 8 | 9 |
| P1, Sarpy Creek | 1.75 | 4 | 3 |
| P4, Kluver E1 | 1.13 | 2 | 4 |
| P7, Diamond Buttes | 0 | 0 | 0 |

TABLE 3.4. LICHEN COVER OF PONDEROSA PINE TREE TRUNKS, COLSTRIP AREA. (Percentage cover/frequency, 10 trees sampled on each site. Site P7 has 0 for all species.)

| Lichen | | P10 | P12 | P14 | P11 | P16 | P13 | P15 | P5 | P3 | P2 | P1 | P4 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Usnea hirta | N | 12.0/10 | 5.9/10 | 5.9/10 | 3.1/10 | 3.9/10 | 2.1/10 | 3.1/10 | 0.9/7 | 1.6/9 | 1.0/8 | 0.4/3 | 0.8/1 |
| | S | 11.1/8 | 2.8/7 | 0.8/6 | 3.1/7 | 0.3/2 | 0.8/3 | 0 | 0 | 0.3/1 | 0.1/1 | 0 | 0 |
| | E | 7.4/9 | 6.6/9 | 3.3/7 | 4.6/9 | 1.1/6 | 3.0/9 | 0.1/1 | 0.3/1 | 0 | 0.1/1 | 0 | 0.1/1 |
| | W | 9.3/10 | 1.8/7 | 0.8/6 | 0.9/5 | 1.1/7 | 0.3/2 | 0.5/4 | 0 | 0.4/2 | 0.3/2 | 0 | 0 |
| Parmelia sulcata | N | 0 | 1.9/5 | 1.8/4 | 0.3/2 | 2.3/4 | 0.3/2 | 0.3/2 | 0.3/2 | 0.4/3 | 0.3/2 | 0 | 0 |
| | S | 0 | 0.1/1 | 0 | 0 | 0.8/1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | E | 0 | 0.4/3 | 0 | 0.3/2 | 0 | 0.3/2 | 0 | 0 | 0 | 0 | 0 | 0 |
| | W | 0 | 0 | 0.3/2 | 0 | 0 | 0 | 0.3/2 | 0 | 0 | 0 | 0 | 0 |
| Parmeliopsis ambigua | N | 0 | 0 | 0.1/1 | 0.3/2 | 0.8/1 | 1.0/3 | 3.5/8 | 3.5/8 | 3.6/5 | 1.4/5 | 0.9/2 | 0.1/1 |
| | S | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | E | 0 | 0 | 0 | 0.1/1 | 0 | 1.0/1 | 0.4/3 | 1.6/3 | 0.3/1 | 0 | 0.1/1 | 0 |
| | W | 0 | 0 | 0 | 0.1/1 | 0 | 0 | 0.4/3 | 0.1/1 | 0.1/1 | 0 | 0.1/1 | 0 |
| Alectoria fuscescens | N | 0 | 0.3/2 | 2.1/7 | 0.1/1 | 0.4/3 | 0.3/2 | 0.6/6 | 0.6/5 | 0.5/4 | 0.1/1 | 0.1/1 | 0 |
| | S | 0 | 0 | 0 | 0.3/1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | E | 0 | 0.1/1 | 0.1/1 | 0.3/2 | 0 | 0.1/1 | 0 | 0.3/2 | 0 | 0 | 0 | 0 |
| | W | 0 | 0 | 0.1/1 | 0 | 0.4/3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hypogymnia physodes | N | 0 | 0.9/2 | 0 | 0 | 0 | 0.9/3 | 0.3/2 | 0.9/2 | 1.4/6 | 0.5/4 | 0 | 0.1/1 |
| | S | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | E | 0 | 0.1/1 | 0 | 0 | 0 | 0 | 0 | 0.1/1 | 0 | 0 | 0 | 0 |
| | W | 0 | 0 | 0 | 0 | 0.1/1 | 0.1/1 | 0.1/1 | 0 | 0.1/1 | 0.1/1 | 0 | 0 |
| Parmelia infumata | N | 0 | 0 | 0 | 0 | 0.1/1 | 0 | 0.3/2 | 0.4/3 | 0.3/1 | 1.0/4 | 0 | 0 |
| | S | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | E | 0 | 0 | 0 | 0 | 0.8/1 | 0 | 0 | 0.1/1 | 0.1/1 | 0.1/1 | 0 | 0 |
| | W | 0 | 0 | 0 | 0 | 0 | 0 | 0.1/1 | 0 | 0 | 0 | 0 | 0 |

(continued)

TABLE 3.4. (continued)

| Lichen | | P10 | P12 | P14 | P11 | P16 | P13 | P15 | P5 | P3 | P2 | P1 | P4 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Lecanora subfusca* | N | 0 | 0 | 0 | 0 | 0 | 0 | 0.1/1 | 0.9/1 | 0 | 2.4/4 | 0 | 0 |
| | S | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | E | 0 | 0 | 0 | 0.1/1 | 0 | 0 | 0.4/3 | 0 | 0 | 0 | 0 | 0 |
| | W | 0 | 0 | 0 | 0 | 0 | 0 | 0.4/3 | 0 | 0 | 0 | 0 | 0 |
| *Cetraria pinastri* | N | 0 | 0 | 0 | 0 | 0 | 0.4/1 | 0 | 0.4/1 | 0 | 0.1/1 | 0 | 0 |
| | S | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | E | 0 | 0 | 0 | 0.1/1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | W | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Parmelia ulophyllodes* | N | 0 | 0.3/2 | 0 | 0 | 0.9/2 | 1.1/3 | 0.1/1 | 0 | 0 | 0 | 0 | 0 |
| | S | 0 | 0 | 0.1/1 | 0.1/1 | 0.1/1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | E | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.1/1 | 0 | 0 | 0 |
| | W | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Letharia vulpina* | N | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.1/1 | 0.1/1 | 0 | 0 |
| | S | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | E | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.1/1 | 0 | 0 | 0 |
| | W | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total epiphyte cover: | | 39.75 | 21.00 | 15.25 | 13.63 | 12.88 | 11.75 | 10.81 | 9.13 | 6.88 | 6.38 | 1.75 | 1.35% |

Tables 3.3 and 3.4 give the epiphytic lichen community data collected and compiled through 1976. Only ten species are present consistently enough to appear in the sampling. The species list includes names of other collected epiphytic lichens.

Respiration rates for Usnea hirta samples for 1975 and 1976 are in Table 3.5; those for Parmelia chlorochroa are in Table 3.6. Results from sulfate sulfur analyses for 1974 and 1975 are in Table 3.7; analyses for 1976 samples have not yet been completed.

Determination of some baseline algal plasmolysis counts have been completed from the P10 site. The plasmolysis values (means ± standard deviation) are: 1975, September, 17 ±2; 1976, March, 3 ±3; July, 11 ±2; July, 9 ±3. Counts are continuing for all other collection.

## DISCUSSION

After the summer of 1975, it appeared that recording only the presence/absence data for epiphytic lichen communities was not sensitive enough to detect and monitor the low-level chronic $SO_2$ stress, despite reports using these criteria from other regions (LeBlanc and Rao, 1975). However, in this area any pollution stress will be newly-introduced and epiphytic growth is slight compared to that in the more humid climates of other studies. Therefore, the more detailed data were acquired during 1976. The marked trees allow for annual repetition of measurements on the sites. Three more sites, closer to the power plant, are being added for analysis beginning in 1977.

Determination of respiration rates is a method sensitive enough to detect adverse pollution effects in U. hirta (Eversman, 1976). However, bacteria populations increased and/or changed in $SO_2$-stressed Parmelia chlorochroa plant bodies. This probably caused the respiration rate of the lichen to be overestimated. Photosynthetic rate would be a more specific indicator of the physiological condition of the lichen plant. This information combined with the plasmolysis counts gives adequate information on the vitality of these lichens. Eventually, the effects observed at the organismic level will be reflected at the community level. Photographs of many communities are also being taken regularly.

Ponderosa pine trees in the moister habitats, with shrubs Symphoricarpos albus, Rhus trilobata, and Ribes spp., generally have the heaviest lichen cover on the trunks and branches (P10, P11, P12). Where the understory is dominated by such species as Yucca glauca and Agropyron spicatum (sites P1, P3, P4, P6, P7), the lichen cover is sparse and restricted to the lower 30 cm of trunks on the north and east sides, if it appears at all. None of the sites in the Custer National Forest required transplants for monitoring purposes.

Mosses, the only bryophytes encountered, have been collected from the grasslands and from the soil and litter under the Ponderosa pines. Verified identifications of these species, and of mosses and lichens collected from the rocks, have not yet been completed.

TABLE 3.5. RESPIRATION RATES OF Usnea Hirta SAMPLES, COLSTRIP AREA, 1975-76. (Respiration rate is given as microliters of $O_2$ consumed/g dry weight/hour, manometrically determined.)

| Site | Collection Date | $\bar{x}$ | 1 s.d. |
|---|---|---|---|
| P1, Sarpy Creek | 9-25-75 | 640* | 40 |
| | 9-14-76 | 658 | 129 |
| P2, Castle Rock | 9-25-75 | 640* | 40 |
| | 9-14-76 | 499 | 86 |
| P3, Kluver NE1 | 9-25-75 | 640* | 40 |
| | 9-15-76 | 633 | 98 |
| P4, Kluver E1 | 9-14-75 | 640* | 40 |
| | 9-15-76 | 573 | 53 |
| P5, D McRae | 9-15-75 | 640* | 40 |
| | 9-15-76 | 827 | 38 |
| P6, Kluver West transplants (from P9, 1974) | 7-16-76 | 669 | 50 |
| P7, Diamond Buttes | 6-24-76 | 744* | 45 |
| P8, Morning Star View, transplants | 9-25-75 | 640* | 40 |
| natives | " | 828 | 135 |
| transplants | 11-20-76 | 661 | 60 |
| P9, Road | 5-01-76 | 563 | 109 |
| | 8-11-75 | 508 | 51 |
| P10, East Otter Creek | 9-25-75 | 640 | 40 |
| | 3-23-76 | 709 | 63 |
| | 6-23-76 | 744 | 45 |
| | 7-15-76 | 463 | 57 |
| | 8-09-76 | 668 | 41 |
| | 9-15-76 | 633 | 53 |
| P11, SEAM 1 | 7-16-75 | 545 | 35 |
| | 7-15-76 | 453 | 19 |
| P12, SEAM 2 | 7-16-17 | 548 | 27 |
| | 7-15-76 | 498 | 48 |
| P13, Home Creek Butte | 7-16-75 | 462 | 55 |
| | 7-15-76 | 486 | 15 |
| P14, 3-Mile, top of ridge | 7-16-75 | 539 | 64 |
| bottom of ridge | " | 528 | 38 |
| top of ridge | 8-09-76 | 667 | 76 |
| bottom of ridge | " | 547 | 40 |
| P15, Fort Howes (transpl from P9) | 5-01-75 | 829 | 58 |
| natives | " | 510 | 213 |
| " | 5-13-76 | 576 | 113 |
| " | 9-15-76 | 470 | 50 |
| transplants | " | 597 | 43 |
| P16, Poker Jim Butte transplants | 9-25-75 | 640* | 40 |
| " | 9-15-76 | 669 | 74 |
| natives | 7-17-75 | 630 | 77 |
| " | 7-16-76 | 610 | 31 |
| " | 9-15-76 | 654 | 52 |

* respiration rate of duplicate material when transplanted from P10 site.

TABLE 3.6. RESPIRATION RATES OF Parmelia chlorochroa SAMPLES, COLSTRIP AREA, 1974-6. (Respiration rate is in microliters $O_2$ consumed/g dry wt/hour, manometrically determined.)

| Site | Collection Date | $\bar{x}$ | 1 s.d. |
|---|---|---|---|
| G1, Hay Coulee | 7-20-74 | 314 | 14 |
| | 8-30-74 | 332 | 43 |
| | 6-26-75 | 215 | 26 |
| | 5-14-76 | 458 | 43 |
| | 7-16-76 | 290 | 19 |
| | 9-14-76 | 274 | 22 |
| G2, McRae Knolls | 9-15-76 | 341 | 21 |
| G3, Kluver West | 7-21-74 | 218 | 16 |
| | 7-07-75 | 349 | 21 |
| | 7-07-75 | 293 | 29 |
| | 7-14-76 | 272 | 25 |
| | 9-14-76 | 301 | 15 |
| G4, Kluver North | 7-21-74 | 342 | 18 |
| | 7-07-75 | 349 | 21 |
| | 7-16-75 | 242 | 4 |
| | 9-14-76 | 262 | 34 |
| G5, Kluver East | 7-21-74 | 227 | 34 |
| | 6-26-75 | 316 | 40 |
| | 7-14-76 | 322 | 26 |
| | 9-15-76 | 404 | 32 |
| | 9-15-76 | 239 | 15 |
| G6, Harvey 1 | 9-15-74 | 339 | 46 |
| | 9-15-74 | 321 | 28 |
| G7, Pasture | 5-01-75 | 337 | 132 |
| | 5-14-76 | 371 | 54 |

TABLE 3.7. SULFUR (AS TOTAL SULFATE) CONTENTS OF *Usnea hirta* AND *Parmelia chlorochroa* SAMPLES COLLECTED IN 1975. (Determined by MSU Soils Testing Laboratory.)

| Site | Collection Date | % sulfur as sulfate | 1 s.d. |
|---|---|---|---|
| *Usnea hirta* | | | |
| P9, Road | 5-01-75 | 0.25 | 0.04 |
| P15, Fort Howes transplants | 5-01-75 | 0.25 | 0.03 |
| P15, Fort Howes natives | " | 0.19 | 0.04 |
| P9, Road | 8-11-75 | 0.26 | 0.04 |
| P3, Kluver NE1 | 9-25-75 | 0.03 | 0.01 |
| P8, Morning Star View | " | 0.19 | 0.08 |
| P13, Home Creek Butte | 7-16-75 | 0.15 | 0.16 |
| P10, East Otter Creek | 7-16-75 | 0.08 | 0.01 |
| P10, East Otter Creek | 9-25-75 | 0.22 | 0.11 |
| *Parmelia chlorochroa* (includes 1974) | | | |
| G1, Hay Coulee | 7-21-74 | 0.26 | 0.02 |
| G3, Kluver West | " | 0.22 | 0.02 |
| G4, Kluver North | " | 0.20 | 0.02 |
| G5, Kluver East | " | 0.26 | 0.02 |
| G6, Harvey | 9-15-74 | 0.23 | 0.01 |
| Taylor Creek | 5-01-75 | 0.17 | 0.02 |
| G7, Pasture | " | 0.19 | 0.03 |

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## APPENDIX

### LIST OF LICHEN SPECIES COLLECTED FROM THE COLSTRIP, MONTANA, AREA; BIG HORN, ROSEBUD, AND POWDER RIVER COUNTIES.

(Collection number(s); site; substrate.)

1. *Acarospora schleicheri* (Ach.) Mass. 565, G1. Soil.
2. *Agrestia hispida* (Mereschk.) Hale & W.Culb. 569, G6; 609, Sarpy Creek; 720, P7; G5. Soil.
3. *Alectoria fuscescens* 684, Upper Indian Creek Divide; 690, P3; 694, P14; 556, Ash Creek. Ponderosa pine base. det. I.M. Brodo.
4. *Caloplaca aurantiaca* (Lightf.) Th.Fr. 547, P6. Decorticated stump.
5. *Candelariella aurella* (Hoffm.) Zahlbr. 710, P16; 744, P11. Rock.
6. *Cetraria pinastri* (Scop.) S. Gray. 548, Ash Creek; 733, P11. Pine tree trunk.
7. *Cladonia pyxidata* (L.) Hoffm. 543, 544 557, G3; 571, G6. Soil.
8. *Cladonia* spp. squamules All sites. Soil.
9. *Collema tenax* (Sw.) Ach. 580, Ash Creek; 581, G4; 582, G1. Soil.
10. *Cyphelium notarisii* 549, P6. Decorticated stump.
11. *Dermatocarpon lachneum* (Ach.) A.L.Sm. 584, G1. Soil.
12. *Evernia mesomorpha* Nyl. 598, P15; 660, P10. Ponderosa pine base.
13. *Fulgensia bracteata* (Hoffm.) Ras. with *F. fulgens* (Sw.) Elenk. 716, P7; 546, 545, G3; 560, G1; 568, G6. Soil.
14. *Hypogymnia physodes* (L.) W. Wats. 538, P15; 539, Ash Creek; 688, P3; 698, P16; 703, P8. Pine tree trunk.
15. *H. bitteri* (Lynge) Ahti. 676, P16. Ponderosa pine trunk.
16. *Lecanora calcarea* (L.) Somm. 608, P16. Rock.
17. *L. chrysoleuca* (Sm.) Ach. 669, P10. Rock.
18. *L. melanophthalma* (Ram.) Ram. 709, P16; 669, P10. Rock.
19. *L. muralis* (Schreb.) Rabenh. 665, P8. Rock.
20. *Lecidea decipiens* (Hedw.) Ach. 566, G6; 567, G1. Soil.
21. *L. marginata* Schaer. 682, Upper Indian Creek; 705, P16; 732, P14. Rock.
22. *L. rubiformis* (Wahlenb. ex. Ach.) Wahlenb. 610, Sarpy Creek; 648, P16; 704, P5; 715, P7; 731, P16. Soil in rock crevices.
23. *Letharia vulpina* (L.) Hue. 654, P6; 597, P15; 689, P3. Pine tree trunks
24. *Omphalodiscus virginis* (Schaer.) Schol. 693, P8. Rock.
25. *Parmelia chlorochroa* Tuck. G3; 559, G1; 653, P1; 656, G5; 658, Sarpy Creek. Soil.
26. *P. infumata* Nyl. 554, Ash Creek; 674, P16; 738, 745, P11; 729, P16. Pine tree trunk.
27. P. *elegantula* (Zahlbr.) Szat. 729, P16. Pine tree trunk.
28. *P. lineola* Berry 666, 671, P10. Rock.
29. *P. subdecipiens* Vain. 562, P15; 652, P1. Rock.
30. *P. subolivacea* Nyl. 553, 557, Ash Creek. Pine tree trunk, branches.
31. *P. sulcata* Tayl. 552, Ash Creek; 675, P16; 696, P14; 735, P11. Pine tree trunk.

32. P. ulophyllodes (Vain.) Sav. 563, Ash Creek; 564, P15; 695, P14; 699, P16. Pine trunk.
33. Parmeliopsis ambigua (Wulf.) Nyl. 605, P3; 672, P10; 685, Upper Indian Creek; 691, P3; 734, P11. Base of pine trees.
34. Physcia caesia (Hoffm.) Hampe 558, P15. Rock.
35. Peltigera canina (L.) Willd. 579, P15. Soil.
36. P. rufescens (Weis.) Humb. 692, P3. Soil.
37. Rinodina confragosa (Ach.) Koerb. 702, P1. Rock.
38. Squamarina lentigera (G.Web.) Poelt. 570, G1; 718, P7. Soil.
39. Toninia caeruleonigricans (Lightf.) Th.Fr. 714, P7. Soil.
40. Usnea hirta (L.) Wigg. 603, Whitetail Guard Station; 550, Ash Creek; 572, P15; 686 Upper Indian Creek.
41. Umbilicaria torrefacta (Lightf.) Schrad. 740, P11. Rock.
42. Umbilicaria vellea (L.) Ach. 608, Sarpy Creek. Rock.
43. Verrucaria sp. 706, P16. Rock.
44. Xanthoria elegans (Link.) Th.Fr. 609, P10; 707, P16. Rock.
45. X. polycarpa (Ehrh.) Oliv. 673, P15. Shrub branches.

## SECTION 4

# INVESTIGATION OF THE IMPACT OF COAL-FIRED POWER PLANT EMISSIONS UPON THE DISEASE/HEALTH/GROWTH CHARACTERISTICS OF PONDEROSA PINE-SKUNKBUSH ECOSYSTEMS AND GRASSLAND ECOSYSTEMS IN SOUTHEASTERN MONTANA

by

C. C. Gordon, P. C. Tourangeau, and P. M. Rice

## INTRODUCTION

This portion of the EPA-CERL (Corvallis Environmental Research Laboratory, Oregon)-sponsored studies was initiated to ascertain and quantify the impact of coal-fired power plant emissions upon the two major ecosystems of the Colstrip area of southeastern Montana: (1) Ponderosa pine-skunkbush (Pinus ponderosa-Rhus trilobata) and (2) cool season-short grass. Before the two, 350 MW capacity coal-fired power plants were constructed and began operations at Colstrip, air monitoring studies by federal and state agencies, the utility companies, and private research institutes established that ambient air in the Colstrip area was as pristine and free of phytotoxic gases as any area in the contiguous United States thus far studied (Northern Cheyenne Research Project, 1976). Because of the pristine nature of this area of Montana, it was hypothesized that if the baseline characteristics of the chemical, physical, and growth/health/disease parameters of indigenous flora and fauna species could be established and quantified before the power plants began operations, many future direct and indirect impacts of the atmospheric emissions upon these species could be measured and quantified. To test this hypothesis, several study objectives were carried out. These encompassed the establishment of:

(1) Baseline levels of sulfur and fluoride concentrations in the dominant indigenous plant species of both the ponderosa pine-skunkbush and cool season-short grass ecosystems;

(2) baseline growth/health/disease parameters of ponderosa pine foliage;

(3) baseline insect population and insect damage parameters for ponderosa pine of the area;

(4) baseline fungal population and damage to selected indigenous plant species; and

(5) baseline physical (pH) and chemical parameters of precipitation.

These baseline parameters and characteristics were used to study species of flora and fauna collected from permanently established ponderosa pine-skunkbush sites located at varying distances and directions from Colstrip during the 1974 and 1975 growing seasons.

After the 1974 and 1975 baseline studies on the ponderosa pine-skunkbush sites were completed (see EPA-CERL 1976 Second Interim Report, EPA-600/3-76-013), some of the earlier study objectives were deleted (i.e., fungal populations and host-parasite relationships on indigenous plant species) and others were modified (i.e., the number of ponderosa pine-skunkbush sites and precipitation chemistry studies). The modification or deletion of those original objectives were initiated to allow concentration on those objectives which more adequately tested the original hypothesis.

During 1975 and 1976, air monitoring by the Montana Department of Health and Environmental Sciences (DHES) Air Quality Bureau at their monitoring site 4 km southeast of Colstrip disclosed that $SO_2$, $NO_2$, $O_3$, and $F^-$ concentrations in the ambient air were all substantially below both federal and Montana state standards for these pollutants (Maughan, 1977). Wind speed and direction data collected by personnel of the DHES Air Quality Bureau at their Colstrip site disclose that the prevailing winds during the growing season (May to September) are to the southeast and east and to the west and northwest, and average between two to six miles per hour.

Since September 1975, when Colstrip Unit 1 went on line, and July 1976, when Unit 2 started up, these two units have operated at an average megawatt capacity of 1/3 (33%) to 2/5 (40%) of their total rated capacity of 700+ MW. The estimated atmospheric emissions of the Colstrip units when operating at 1/3 and 2/5 capacity are presented in Table 4.1 below.

TABLE 4.1. ESTIMATED ATMOSPHERIC EMISSIONS OF COLSTRIP UNITS.

| | Unit 1 & 2 at 1/3 capacity | Unit 1 & 2 at 2/5 capacity | Unit 1 & 2 at full capacity |
|---|---|---|---|
| $SO_2$ (tons/yr) | 6,046 | 7,256 | 18,142 |
| $NO_x$ (tons/yr) | 6,920 | 8,304 | 20,760 |
| Particulate (tons/yr) | 536 | 644 | 1,612 |
| Fluoride (lbs/yr) | 4,666 | 5,600 | 14,000 |

Source: Montana Department of Health and Environmental Sciences, 1975

## MATERIALS AND METHODS

### COLLECTION OF VEGETATION

Five ponderosa pine-skunkbush sites located east to south of Colstrip at distances of 5 to 80 km were utilized during both the 1975 and 1976 study periods. The locations of each of these five sites are listed in Table 4.2.

TABLE 4.2. LOCATIONS OF FIVE PONDEROSA PINE-SKUNKBUSH SITES.

| Ponderosa Pine-Skunkbush Sites | Location | Distance and Direction from Colstrip |
|---|---|---|
| Plot S-3 | T2S, R41E, Sec NE 12 | 26 km SSE |
| Plot S-5 | T6S, R44E, Sec SE 8 | 66 km SSE |
| Plot SE-2 | T1S, R42E, Sec SW 1 | 16 km SSE |
| Plot SE-4 | T47E, R47E, Sec NW 19 | 56 km ESE |
| Plot E-1 | T2N, R42E, Sec SW 29 | 5 km E |

Ponderosa pine foliage was collected during the 1975 field season from these plots for fluoride and sulfur analysis, evaluation of seven selected foliar pathologies, determination of percent needle retention, and measurement of needle length and fascicular cross-sectional area.

Each of the five plots consisted of ten permanently marked trees. Based on tree height and diameter at breast height (DBH), five of the trees were classified as "older" and five were classified as "younger." From each of these ten trees, four branches were removed from the upper third of the crown and four branches from the lower third of the crown from the side of the trees facing the Colstrip steam generating complex. The branches from each tree were placed in plastic sacks with appropriate identification of tree number and crown position and transported to the field laboratory or to the Environmental Studies Laboratory at the University of Montana for subsequent preparation for chemical analyses, pathological evaluations, and measurements.

The percent needle retention for 1972, 1973, and 1974 year's foliage was determined for each internode from each branch for the respective categories of crown position and tree age by counting all fascicular scars on each internode, then removing all retained fascicles, counting them, and computing the percent needle retention as shown:

$$\left(\frac{\text{fascicles present}}{\text{fascicles present + number of fascicular scars}}\right) \times 100$$

The mean percent needle retention for each year's internode was computed, and subsequent data analyses used these basic values.

One hundred fascicles were randomly selected from the group of retained fascicles from each internode, the fascicular sheaths were removed, and one needle was randomly selected from each fascicle for pathological evaluation. Ten fascicles were also selected for measurement of fascicular cross-sectional area. After evaluation, 25 needles were randomly selected for measurement of needle length.

Each needle was evaluated for the presence of seven pathologies: (1) percent basal necrosis, (2) percent basal scale, (3) percent defoliator, (4) percent tip burn, (5) percent total necrosis, (6) percent mottled needles, and (7) percent healthy needles. Percent healthy needles is the percentage of total needles without any visible pathology whatsoever and, as such, are completely green. Percent total necrosis is an estimate of the total necrotic surface area of the 100 needles. This category includes chlorosis or yellowing. The percent occurrence of each pathology was computed, and all data analyses used these derived values.

The method of sampling results in the partitioning of nine categories of tree age and crown position within each plot for each year's foliage is as follows:

Upper Crown - Younger Trees
Upper Crown - Older Trees
Upper Crown - All Tree Ages
Lower Crown - Younger Trees
Lower Crown - Older Trees
Lower Crown - All Tree Ages
All Crown Positions - Younger Trees
All Crown Positions - Older Trees
All Crown Positions - All Tree Ages

These categories permit a detailed analysis of within-plot and between-plot variability as a function of duration of foliage exposure. Studies have shown that sulfur and fluoride are partitioned in greater concentrations in the taller dominant vegetation at a site which is impacted by the emissions of a coal-fired power plant and which includes vegetation species similar to those discussed here (Gordon et al., 1977); other studies have demonstrated that fluoride is partitioned in the upper crown of conifers. Any investigation of the effects of coal-fired power plant emissions upon ponderosa pine sites in southeastern Montana requires a background preoperational analysis of the partitioning of fluoride and sulfur, pathologies associated with air pollution, needle length, and fascicular cross-sectional area in the upper and lower crowns of ponderosa pine.

Understory species of grass, shrubs, and forbs were collected at the five ponderosa pine-skunkbush sites during both the 1975 and 1976 summer collection periods. A list of the understory species collected is presented in Table 4.3. At each site, at least eight to ten different species of understory were collected where possible. Collection of leaf foliage from each understory species was obtained from a minimum of three plants and these were lumped and tagged with the collection number, placed in plastic sacks, and brought back to the laboratory for chemical analysis.

Needles manifesting the various needle pathologies (i.e., mottling, weevil, fungal damage) were selected for histological studies. Histological preparation and staining procedures have been described by Jones et al. (1965). Photomicrographs were taken with the use of a Reichert Zetopan phase microscope adapted with Leitz cameras.

TABLE 4.3. UNDERSTORY SPECIES.

| Scientific | Common |
|---|---|
| Artemisia cana | Silver Sage |
| Gutierrezia sarothrae | Broom Snakeweed |
| Prunus virginiana | Chokecherry |
| Rhus trilobata | Skunkbush |
| Festuca idahoensis | Idaho Fescue |
| Agropyron spicatum | Bluebunch Wheatgrass |
| Artemisia ludoviciana | Prairie Sage |
| Balsamorhiza sagittata | Arrowleaf Balsamroot |
| Andropogon scoparius | Little Bluestem |
| Lupinus sp. | Lupine |
| Psoralea sp. | Scurf-pea |
| Yucca glauca | Yucca |
| Aristida longiseta | Red Three Awn |
| Stipa comata | Needle-and-thread |
| Vicia sp. | Vetch |
| Artemisia frigida | Fringed Sage |
| Chrysothamnus nauseosus | Common Rabbit-brush |
| Juniperus scopulorum | Rocky Mountain Juniper |
| Artemisia tridentata | Big Sage |

## CHEMICAL ANALYSIS OF VEGETATION

The methods of chemical analyses for sulfur and fluoride have been reported in detail elsewhere (Kay et al., 1975; EPA, 1976). Therefore, these procedures are summarized here.

Vegetation was dried under forced draft at 90°F, ground in a Wiley mill to pass 30 mesh, and stored in capped plastic vials. For fluoride analysis, 0.50 g of dried ground plant material was slurried with CaO, charred under infrared, and ashed overnight at 600°C in a muffle furnace. The ash was dissolved in 30% $HClO_4$, made to 100 ml with 50% TISAB, and the fluoride activity was determined with an Orion specific ion electrode. The calculations for determining the fluoride concentration in the original plant sample were computerized.

Sulfur determinations were carried out using a Leco induction furnace. Before the data analysis, each derived variate (percents basal necrosis, basal scale, needle retention, tip burn, mottled needles, total necrosis, and healthy needles) was coded by the arcsine transformation for percentages where:

$$\text{coded variate} = \text{arcsine} \sqrt{\frac{\text{variate}}{100}} \times 57.29578$$

All coded variates and sample statistics were decoded for the presentation of results.

## RESULTS

### DATA ANALYSIS OF VEGETATION

The data for both 1975 and 1976 were interrogated by Analysis of Variance (ANOVA). The analyses were performed by the ANOVA computer program of Sokal and Rohlf (1969).*

A four-level nested ANOVA was used because the 1975 data required tests for each variable for: (1) Differences between plots; (2) differences between upper and lower crown positions; (3) differences between the younger and older trees, and (4) differences between the years of foliage (1974, 1973, 1972). The design for the four-level ANOVA is schematically shown in Table 4.4 for any individual plot. In the actual analysis of all variables, the data for each plot were arrayed exactly as in Table 4.4 and all plots were tested simultaneously. In Table 4.4 the highest level, Level 4, is the individual plot. At Level 3 are the two crown positions (upper and lower) within each plot. At Level 2 are the tree ages (younger and older), and at Level 1 are the three years of foliage (1974, 1973, 1972). The lowest level, Level 0, is the individual variates, denoted as 1, 2, 3, 4, 5 to indicate the five samples measured for fluoride content. Since each plot consists of five older and five younger trees, the most fundamental data block in any plot would be the five variates for a particular year's foliage for younger or older trees, from either crown position. As can be seen from this design, tests are performed between the years of foliage, between the tree ages, between the crown positions, and between the plots.

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*The Program, as published in this edition, contains errors. Interested readers should contact the authors of the text for current listings.

TABLE 4.4. THE STRUCTURE OF THE FOUR-LEVEL NESTED ANOVA FOR EACH INDIVIDUAL PLOT, 1975 COLLECTION, BOTH CROWN POSITIONS.

| Level 4 - Plots | Plot | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Level 3 - Crown Positions | Upper | | | | | | Lower | | | | | |
| Level 2 - Tree Ages | Younger | | | Older | | | Younger | | | Older | | |
| Level 1 - Age of Foliage | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Level 0 - Five Individual Observations for Each Variable within Each Age of Foliage | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |

TABLE 4.5. FOUR-LEVEL ANALYSIS OF VARIANCE TABLE FOR FLUORIDE, 1975 COLLECTION.

| Source of Variation | Sums of Squares | Degrees of Freedom | Mean Squares | F Ratio |
|---|---|---|---|---|
| Between the Plots (Level 4) | 29.004 | 4 | 7.251 | 15.031* |
| Between Crown Positions within the Plots (Level 3) | 2.412 | 5 | .482 | .989 |
| Between the Tree Ages within the Crown Positions (Level 2) | 4.876 | 10 | .487 | 1.032 |
| Between the Years of Foliage within the Tree Ages (Level 1) | 18.889 | 40 | .472 | 1.091 |
| Within the Years of Foliage (Level 0) | 103.868 | 240 | .432 | |

*F ratio significant @ $p \leqq .05$

TABLE 4.6. THE STRUCTURE OF THE THREE-LEVEL NESTED ANOVA FOR EACH INDIVIDUAL PLOT, 1976 COLLECTION.*

| | | | | | | |
|---|---|---|---|---|---|---|
| Level 3 - Plots | Plots | | | | | |
| Level 2 - Tree Ages within the Plot | Younger | | | Older | | |
| Level 1 - Age of Foliage within the Tree Ages | 1 | 2 | 3 | 1 | 2 | 3 |
| Level 0 - Five Individual Observations for Each Variable within the Ages of Foliage | 1 | 1 | 1 | 1 | 1 | 1 |
| | 2 | 2 | 2 | 2 | 2 | 2 |
| | 3 | 3 | 3 | 3 | 3 | 3 |
| | 4 | 4 | 4 | 4 | 4 | 4 |
| | 5 | 5 | 5 | 5 | 5 | 5 |

*This design is precisely the same as that used for individual upper or lower crown positions in the 1975 collection.

The results of the four-level ANOVA for fluoride for the 1975 collections are shown in Table 4.5, wherein the levels as detailed in Table 4.4 are listed under Source of Variation. The only level in Table 4.5 which showed a significant effect was Level 4, Between the Plots. The conclusion to be drawn from Table 4.5 is that the means for the five plots are sufficiently different, that they represent different populations as regards fluoride concentrations. Also, it may be concluded that within the plots mean values for the respective crown positions, tree ages, and individual years of foliage would represent the same population.

In 1976, collections were obtained from upper crown positions only—therefore the level supporting upper and lower crown for the ANOVA for 1975 is not needed. The design for the 1976 collection is shown in Table 4.6 as a three-level nested ANOVA*, where Level 3 is the individual plot. At Level 2 are the tree ages within the plots, at Level 1 are the individual years of foliage within the ages, and at Level 0, the individual variates.

The results of ANOVA for fluoride for 1976 are shown in Table 4.7. None of the F ratios are significant, and it may be concluded that fluoride means from plots, tree ages, and years of foliage from the upper crown represent samples from the same population.

TABLE 4.7. ANALYSIS OF VARIANCE TABLE FOR FLUORIDE, 1976 COLLECTION.

| Source of Variation | Sums of Squares | Degrees of Freedom | Mean Squares | F Ratio |
|---|---|---|---|---|
| Between the Plots (Level 3) | 3.912 | 4 | .978 | 2.025 |
| Between the Tree Ages within the Plots (Level 2) | 2.415 | 5 | .483 | 1.532 |
| Between the Years of Foliage within the Tree Ages (Level 1) | 6.303 | 20 | .315 | .819 |
| Within the Years of Foliage (Level 0) | 46.176 | 120 | .384 | |

The 1975 collections from the upper crown and the 1976 collections were compared by a four-level nested ANOVA shown as Table 4.8, where, for brevity, only two plots are shown. At Level 4 are the two collections, at Level 3 are the plots within the collections, at Level 2 are the two tree ages within

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*In 1975 collections, ANOVA was performed on the upper and lower crown data separately and used this design.

TABLE 4.8. THE STRUCTURE OF THE FOUR-LEVEL NESTED ANALYSIS OF VARIANCE FOR BOTH 1975 AND 1976 COLLECTIONS, UPPER CROWNS.

| Level 4 - Collections | 1975 Collection | | | | | | | | | | | | 1976 Collection | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Level 3 - Between the Plots within Collections | Plot 1 | | | | | | Plot 2 | | | | | | Plot 1 | | | | | | Plot 2 | | | | | |
| Level 2 - Between Tree Age within the Plots | Younger | | | Older | | | Younger | | | Older | | | Younger | | | Older | | | Younger | | | Older | | |
| Level 1 - Between the Ages of Foliage within the Tree Ages | 74 | 73 | 72 | 74 | 73 | 72 | 74 | 73 | 72 | 74 | 73 | 72 | 75 | 74 | 73 | 75 | 74 | 73 | 75 | 74 | 73 | 75 | 74 | 73 |
| Level 0 - Five Individual Observations for Each Variable within the Ages of Foliage | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |

the plots within the collections, at Level 2 are the two tree ages within the plots, at Level 1 are the years of foliage, and at Level 0 are the five variates for each year of foliage.

The results of the 1975 and 1976 fluoride analyses are shown in Table 4.9. The F ratios for Level 4 (Between Collections) and Level 3 (Between the Plots within the Collections) are significant at $p \leqq .05$. It may be concluded that mean values for fluoride from the two collections would be sufficiently different such that they would represent samples from different populations; similarly fluoride values from the plots represent samples from different populations. Any level which showed effects significant at $p \leqq .05$ were further interrogated by t-tests.

## HISTOLOGICAL STUDIES

Six plates of photomicrographs depicting three of the needle tissue pathologies being studied on ponderosa pine foliage were prepared for this report. Other pine foliage pathologies caused by insects are presented by Dr. Bromenshenk in his portion of this report.

### Needle Mottling

Air pollution studies have demonstrated that the macroscopic symptoms of conifer needle mottling are a typical host manifestation to phytotoxic gases. However, mottling of ponderosa pine needles can and does occur in pristine areas where no or very low concentrations of phytotoxic gases are present. The tissue pathology of mottling is first manifested in the mesophyll cell beneath the rows of stomatal opening in localized areas of the needles. As illustrated in Plate 1, which shows both cross and longitudinal sections of mottled pine needles, the cell walls of the mesophyll cells beneath the stomatal openings are destroyed in some cases and the surrounding cells then lose their chlorophyll (photo D, Plate 1). In other cases, the mesophyll cells beneath the stomatal openings lose their chlorophyll but retain intact cell walls which lose none of their form (photo B, Plate 1).

Histological studies of mottled needles from the ponderosa sites in the Colstrip area have not established any association thus far with either fungal or insect infestation. Consequently, it appears that the needle mottling recorded in this study is being caused primarily by abiotic factors such as heat (i.e., concentration of the sun's rays by water droplets along stomatal rows).

### Basal Necrosis and Scale

During the 1974 and 1975 study periods, there was a strong possibility that needle tissue pathologies occurring beneath or at the apical opening of the fascicular sheath were caused by both biotic (insects) and abiotic (acidic solutions) causal agents because there were two major macroscopic symptoms involved with these pathologies. These two macrosymptoms were classified as basal needle necrosis and basal needle scale. While both symptoms are associated with necrotic tissues, basal scale is associated only with tissue swelling (hypertrophy) and, in many cases, with the splitting of the epidermal tissues on the swollen area of the needle. Although the authors are not

TABLE 4.9. ANALYSIS OF VARIANCE TABLE FOR FLUORIDE IN BOTH 1975 AND 1976 COLLECTIONS, UPPER CROWNS.

| Source of Variation | Sums of Squares | Degrees of Freedom | Mean Squares | F Ratio |
|---|---|---|---|---|
| Between Collections (Level 4) | 23.914 | 1 | 23.913 | 7.557* |
| Between the Plots within the Collections (Level 3) | 25.314 | 8 | 3.164 | 5.925* |
| Between the Tree Ages within the Plots (Level 2) | 5.340 | 10 | .534 | 1.731 |
| Between the Years of Foliage within the Tree Ages (Level 1) | 12.335 | 40 | .308 | .784 |
| Within the Years of Foliage (Level 0) | 94.360 | 240 | .393 | |

*F ratios are significant @ $p \leq .05$

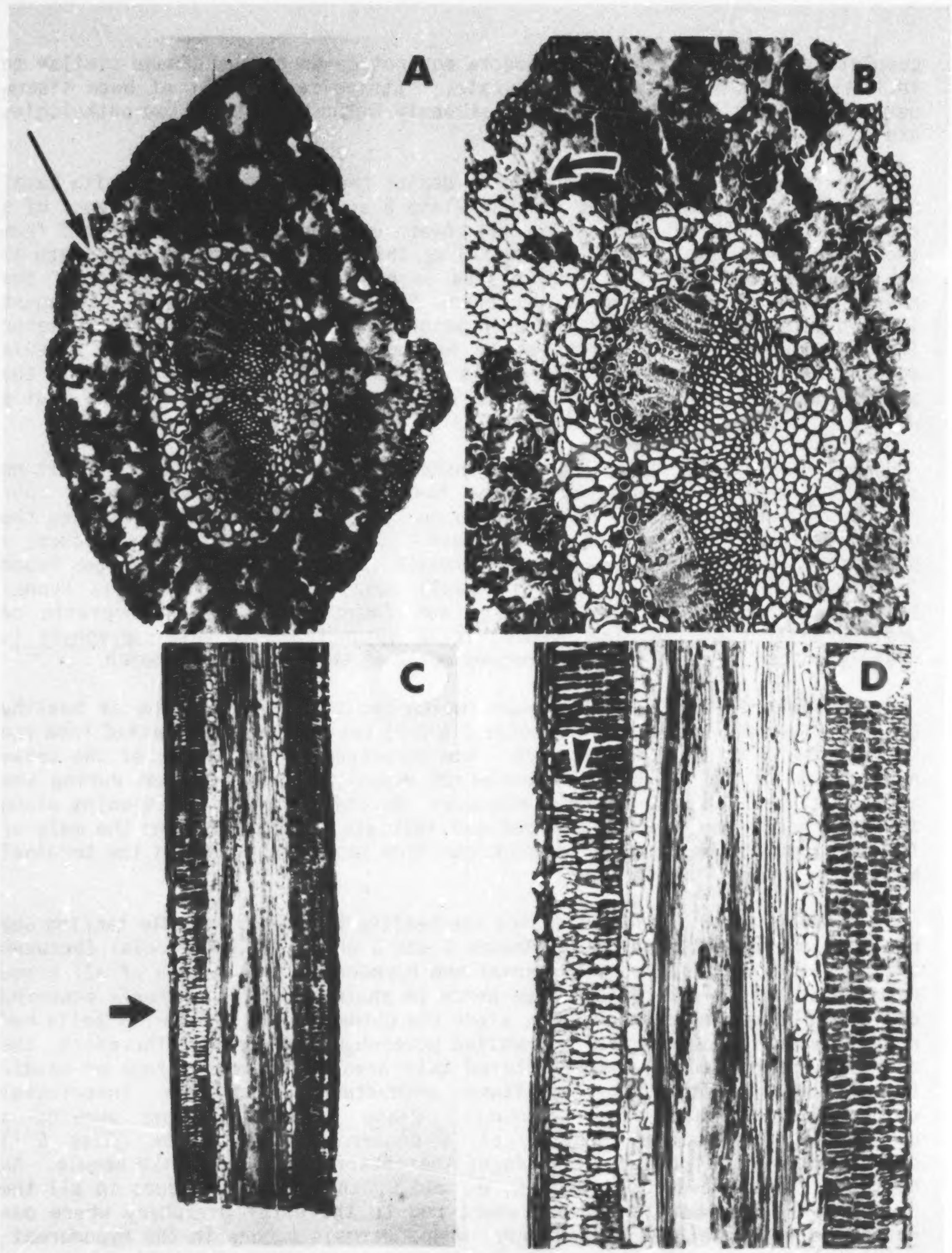

PLATE 1

completely convinced that some insects may not cause tissue damage similar to that classified as basal needle scale, histological studies of both tissue necroses during 1976 and early 1977 strongly indicate that the two pathologies are caused by abiotic agents.

Photomicrographs were prepared to depict the damage associated with basal needle necrosis (Plates 2 to 4). On Plate 2 are three photomicrographs of a cross section of the needle base and sheath of ponderosa pine collected from the McRae pine site (SE-2). In photo B of this plate, one notes that there is no endodermal tissue (see circled area on photo) on the dorsal side of the needle where tissue necrosis occurred. This means that the causal agent induced this damage before the parenchymatous cells in this area differentiated into endodermal cells during early morphogenesis of the needle. Needle morphogenesis of ponderosa pine takes approximately three months after the spring bud break-candle stage period, and thus it is during this time that a needle is damaged by the causal agent.

Both saprophytic fungi and small insects (see Bromenshenk's 1977 report on insects) are common residents of the fascicular sheath area. In all four photographs on Plate 2 one notes the saprophytic fungus Phylctaena sp. on the outer cataphylls of the fascicular sheath. This saprophyte, which produces a pycnidial fruiting body (see arrow in photo D), is the most common fungus found in these histological studies of needle basal tissues. Since its hyphal filaments (vegetative structure) are not found in either the necrotic or healthy tissues of the pine needle, it is suspected that this saprophyte is restricted to the dead sclerenchymatous cells of the fascicular sheath.

On Plate 3 are photomicrographs which depict cross sections of healthy (photos A and B) and necrotic (photos C and D) basal needles collected from the Kluver pine site (E-1) during 1976. The structure in the middle of the three needle set is the dwarf shoot bud which usually remains dormant during the entire life of the pine needles. However, depending on its positioning along the internode, the dwarf shoot bud can initiate growth and form the male or female cones. Also, dwarf shoot buds can form lateral branches if the terminal bud of the branch is destroyed.

Photos A and B of Plate 3 depict the healthy tissue of a needle lacking any basal tissue or cell necrosis. Photos C and D depict an interfacial (between the needle) necrosis of the epidermal and hypodermal tissue area of all three needles of this needle bundle (see arrow in photo C). This necrosis occurred during early needle morphogenesis, since the epidermal and hypodermal cells had not differentiated from the thin-walled parenchymatous cells. Therefore, the causal agent(s) of the damage entered this area at an early stage of needle tissue development. To facilitate understanding of this interfacial epidermal-hypodermal tissue necrosis, Plate 4 was prepared showing a peridermal longitudinal section of a ponderosa pine needle (Site E-1) demonstrating basal tissue necrosis of the interfacial area of the needle. As depicted (see arrows) in photos A, B, and C, the necrosis occurs in all the hypodermal cells from the dwarf shoot bud to the outer periphery where one notes the epidermal one-cell layer. When necrosis occurs in the hypodermal-epidermal tissues of the needle, easy entry to the mesophyll tissue is available to both parasitic or saprophytic fungi. However, this rarely occurs

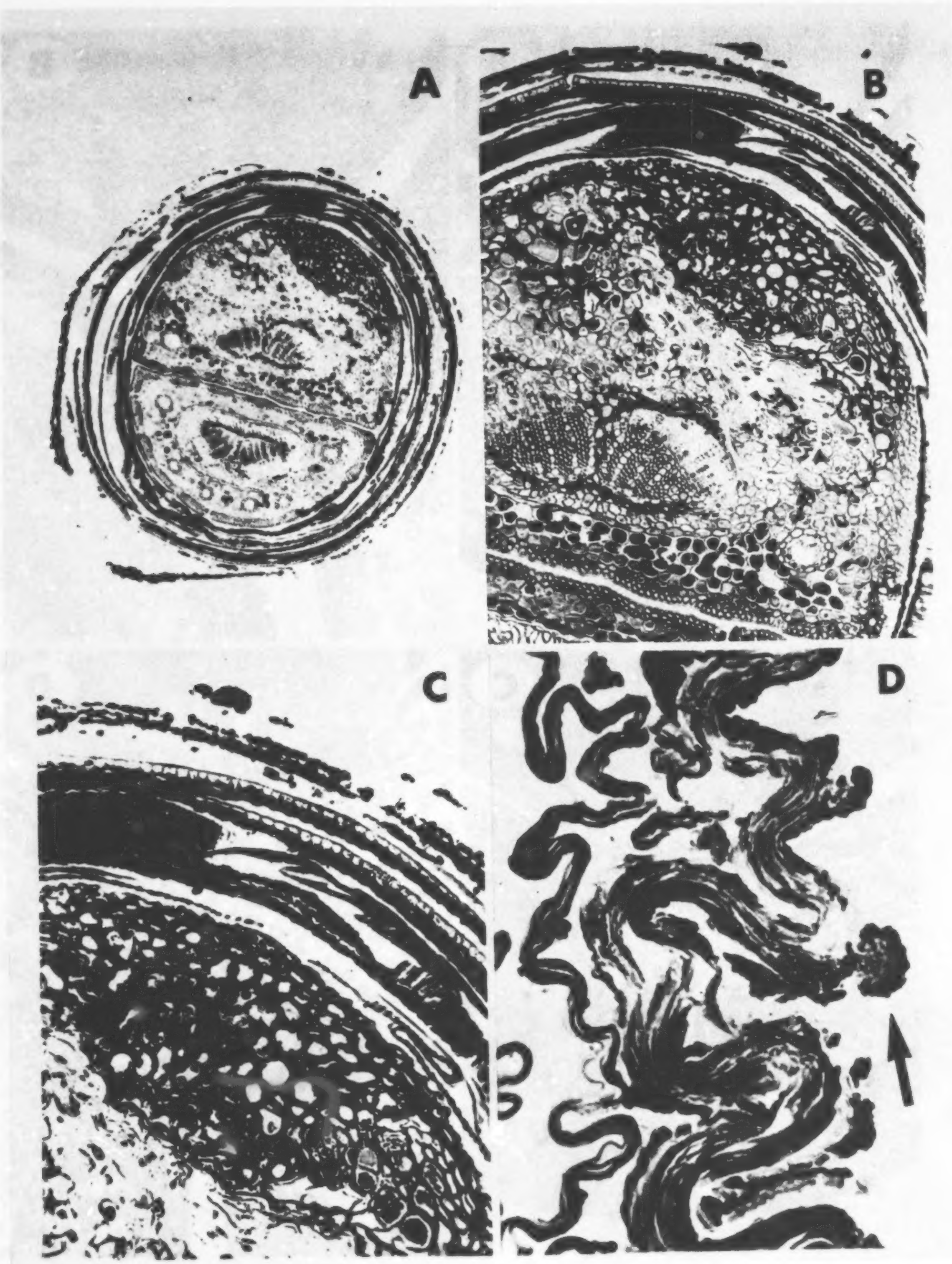

PLATE 2

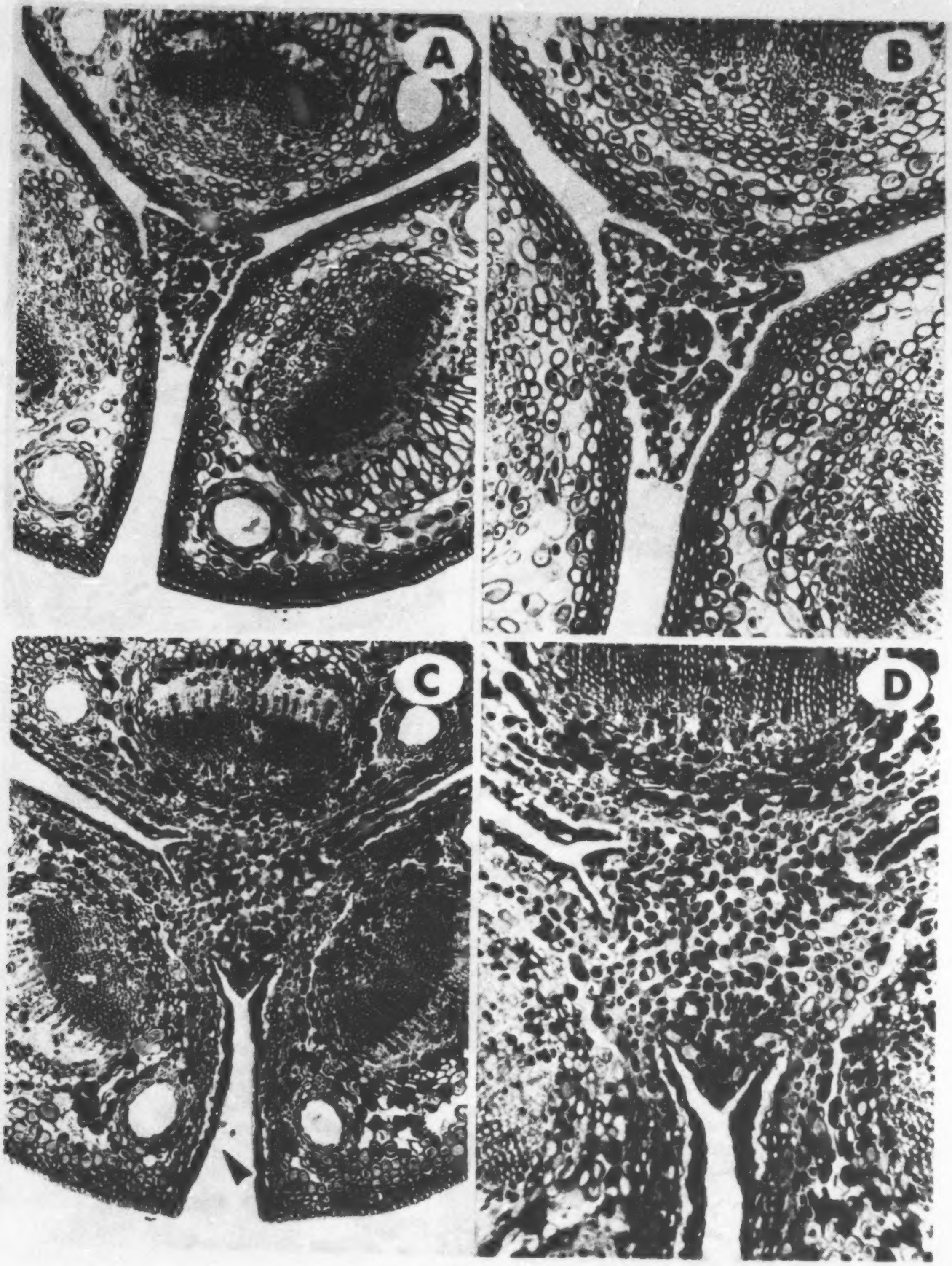

PLATE 3

and there is an extreme dearth of either type of fungi in needles manifesting basal needle necrosis.

One notes in photos A, C, and D of Plate 4 that the dwarf shoot bud is composed entirely of thin-walled parenchymatous cells and that no cell necrosis is occurring in the apical portion of this bud (photo D shows a different section of the same bud). Furthermore, one notes that cellular necrosis is occurring at the base of the dwarf shoot bud, which strongly suggests that the causal agent in this case is lodged only at the base of the bud during the period of penetration for these cells. The photographs on both Plate 3 and Plate 4 strongly support our theory that abiotic causal agents are responsible for basal needle necrosis.

On Plate 5 are photomicrographs of cross sections of basal needle areas manifesting various stages of basal scale. In photo A, the swelling beneath the necrotic epidermal and hypodermal tissues is caused by the production of excessive parenchymatous cells (hyperplasia); if these cells had developed normally during early needle morphogenesis they would have differentiated into mesophyll cells containing chloroplasts. In photo B, the needle cross section depicts no external tissue necrosis but is manifesting the hypertrophy of mesophyll cells and the symptoms of basal scale. One notes that the mesophyll cells adjacent to the hypodermis (see arrow) are undergoing cell enlargement (hypertrophy) but not hyperplasia. In both photos C and D, excessive hypertrophy of mesophyll cells has caused the splitting of the hypodermal and epidermal layers in the basal needle area.

The last plate (Plate 6) depicts cross sections of a ponderosa pine needle manifesting basal necrosis (photos A and B) and a longitudinal section of a ponderosa pine needle manifesting basal scale (photos C and D). As can easily be discerned from these two tissue pathologies, the necrotic cells of the "basal necrosis" symptom do not undergo hypertrophy prior to dying (photos A and B), while hypertrophy of mesophyll cells does occur prior to death in the "basal scale" symptom. Photos A and B of this plate also demonstrate (as photos A and B, Plate 2) that the causal agent caused the damage to the basal needle tissues before formation of the endodermal cells in the area of damage (see arrow in photo B).

## 1975 DATA ANALYSIS

As previously mentioned in the introduction section of this report, each of the 12 pine foliage characteristics being measured is expected or hypothesized to change if and when the atmospheric emissions of coal-fired power plants impact the permanent ponderosa pine-skunkbush sites. The results of the 1975 ponderosa pine-skunkbush studies are presented first so that the reader realizes where the similarities and significant differences within the 12 characteristics occurred before any coal-fired power plants went on-line at Colstrip. After the 1975 data, the results of the 1976 data are presented, and the variability between the 1976 data and 1975 data are then compared utilizing four-level Analysis of Variance (ANOVA).

The data for the 12 growth/health/disease characteristics from both crown positions and the upper and lower crown positions separately from 1975 were

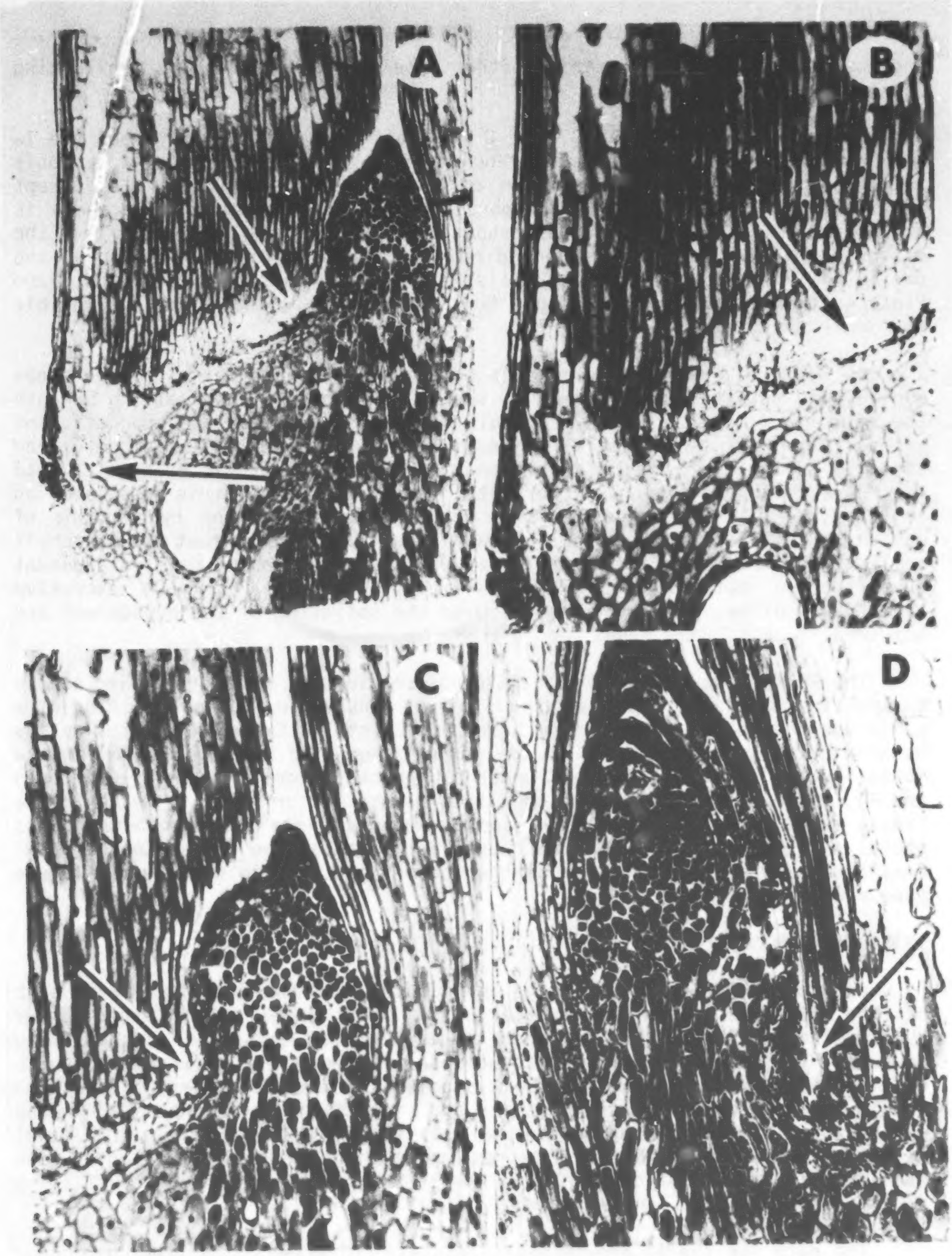

PLATE 4

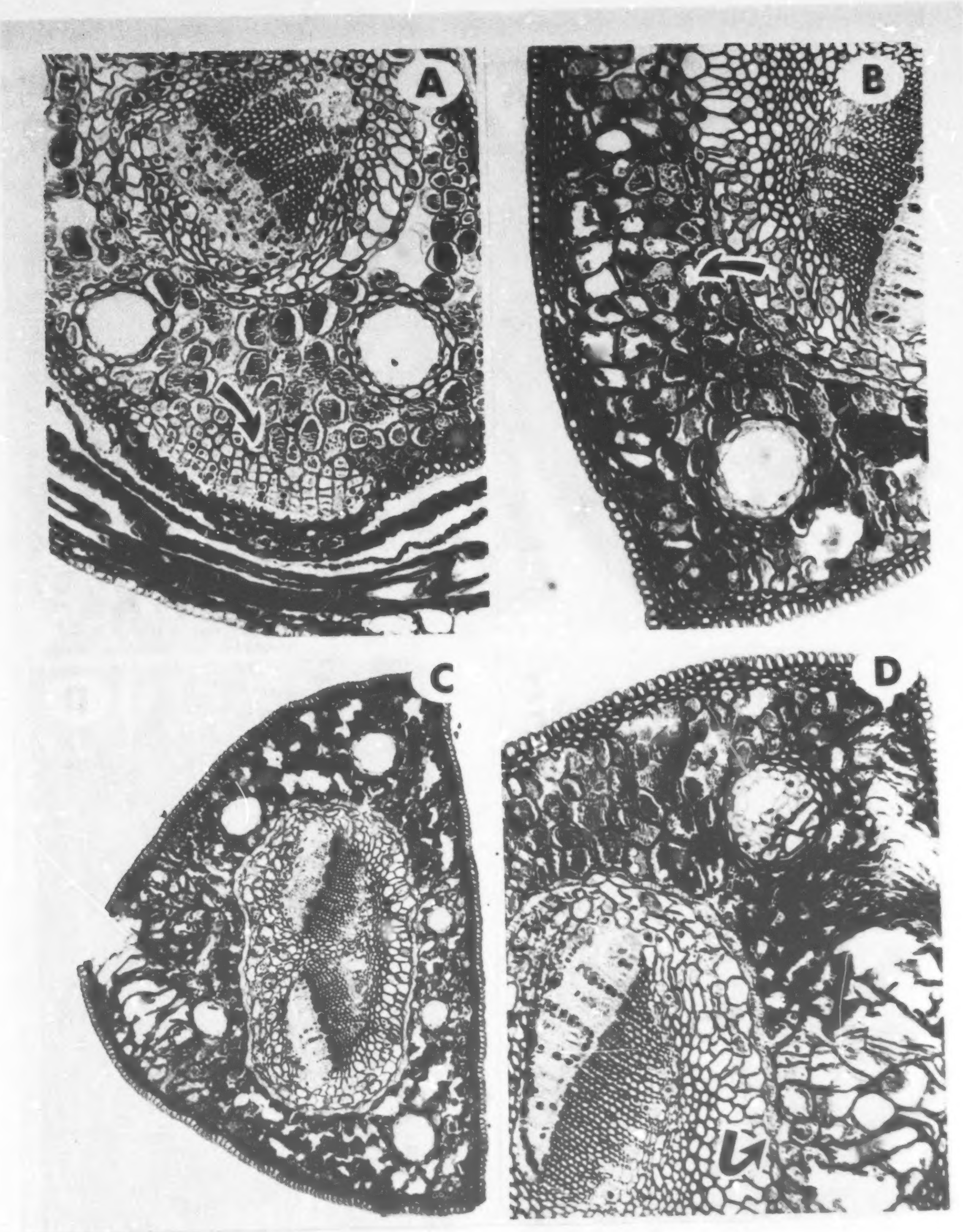

PLATE 5

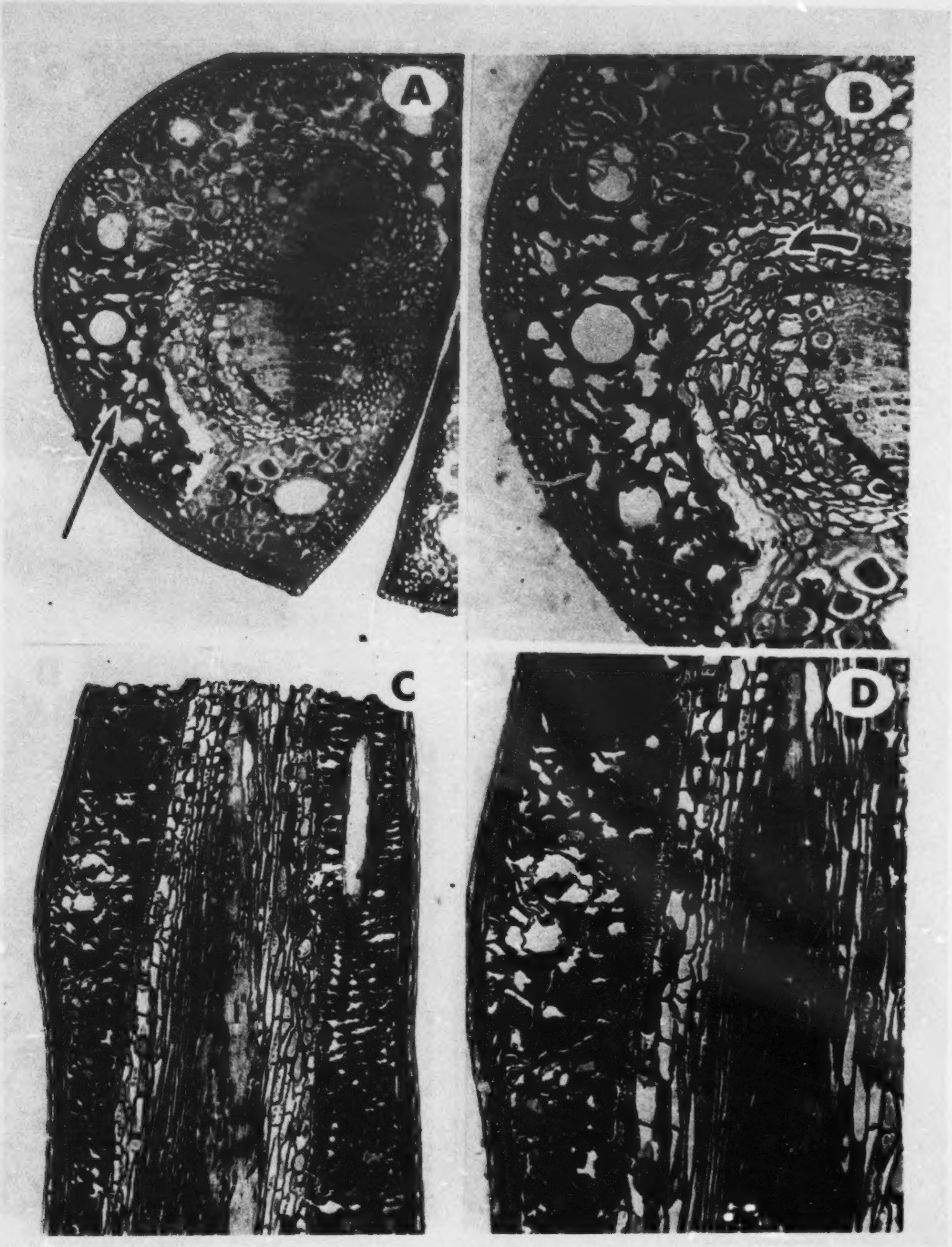

PLATE 6

interrogated by nested ANOVA. These ANOVA designs were previously outlined under Materials and Methods.

The F ratios and the percent variance components for all 12 characteristics from the four-level nested ANOVA, employing both crown positions, are shown on Table 4.10. The F ratios and percent variance components from the ANOVA for upper crown positions are shown on Table 4.11, and those for lower crown positions are arrayed in Table 4.12. The data on these three tables are summarized in Table 4.13, and significant treatment effects ($p \leqq .05$) of any of the 12 characteristics are indicated by asterisks (*).

Inspection of Table 4.10 shows that all growth/health/disease characteristics have significant differences between the plots except for cross-sectional area. The cross-sectional area, however, was the only one which showed a significant treatment effect between the crown positions within the plots (Table 4.10, Level 3). Sulfur levels, cross-sectional area, percent total necrosis, and percent mottled needles showed significant treatment effects due to tree age within the two crown positions (Table 4.10, Level 2). Percent needle retention, both basal needle pathologies, percent tip burn, and percent healthy needles showed significant treatment effects between the three different years of foliage within given tree ages (Table 4.10, Level 1).

As mentioned, Table 4.11 shows the F ratios and percent variance components for 1975 data from the upper crown. Seven of the 12 measured characteristics (i.e., fluoride, cross-sectional area, needle length, percent basal necrosis, percent defoliator, percent tip burn, percent mottled needles) for the upper crown position show significant treatment effects between the five plots (Table 4.11).

Treatment effects due to tree age were found for sulfur, percent total necrosis, and percent mottled needles in the upper crown foliage as was found in the ANOVA for both crown positions (Table 4.10) but not for needle cross-sectional area (Table 4.10, Level 2). Significant treatment effects due to year of foliage of the upper crown are present for percent basal scale, percent basal necrosis, percent tip burn, and percent healthy needles, but not for percent needle retention as was found in the ANOVA using both crown positions (Table 4.10, Level 1).

Table 4.12 contains data obtained from three-level nested ANOVA of the 12 growth/health/disease characteristics of foliage for the 1975 data from the lower crown position. Five of the seven foliage characteristics which had significant treatment effects in the upper crown between plots (Table 4.11, Level 3) showed significant treatment effects in the lower crown; cross-sectional area and percent basal necrosis were not significant at this level (Level 3). A treatment effect due to tree age (Table 4.12, Level 2) was present only for needle cross-sectional area. Percent needle retention, the two basal needle pathologies, percent tip burn, and percent healthy needles showed significant treatment effects due to years of foliage (Table 4.12, level 1) as was found in the ANOVA using both crown positions (Table 4.10, Level 1). The data on Tables 4.10, 4.11, and 4.12 at Level 0 demonstrate that more than 50 percent of the total variance was found within the individual year's foliage.

TABLE 4.10. SUMMARY OF F RATIOS AND % VARIANCE COMPONENTS FOR THE INDICATED LEVELS FOR ALL VARIABLES FROM THE 1975 COLLECTION, BOTH CROWN POSITIONS.

| | Level 4 | | Level 3 | | Level 2 | | Level 1 | | Level 0 |
|---|---|---|---|---|---|---|---|---|---|
| | Between Plots | | Between Crown Positions Within Plots | | Between Tree Ages Within Crown Position | | Between Years Foliage Within Tree Ages | | Residual |
| Variable | F Ratio | Percent Variance Component | F Ratio | Percent Variance Component | F Ratio | Percent Variance Component | F Ratio | Percent Variance Component | Percent Variance Component |
| Fluoride (ppm) | 15.0313* | 20.3 | .9893 | .17 | 1.0325 | .18 | 1.0912 | 1.4 | 77.9 |
| Sulfur (ppm) | 15.6357* | 23.7 | .4764 | -5.6 | 4.0962* | 8.2 | .4883 | -8.3 | 82 |
| Area (mm$^2$) | .3812 | -12.7 | 6.4182* | 19.2 | 3.0008* | 5.4 | .4092 | -11.7 | 99.8 |
| Needle length (mm) | 8.1780* | 28.1 | 2.7015 | 5.3 | 1.2374 | 1.1 | 1.1608 | 2.0 | 63.3 |
| % Needle Retention | 6.0267* | 5.5 | .7143 | 1.7 | .7599 | -2.4 | 1.8939* | 14.4 | 80.7 |
| % Basal Necrosis | 6.4512* | 1.0 | 2.2886 | 9.3 | .1746 | -12.8 | 3.3341* | 32.5 | 69.8 |
| % Basal Scale | 25.1439* | 7.0 | .2011 | 2.5 | .5748 | -6.0 | 3.1461* | 28.9 | 67.4 |
| % Defoliator | 26.5435* | 37.2 | .7619 | -.77 | 1.5119 | 2.5 | 1.2710 | 3.1 | 57.9 |
| % Tip Burn | 559.6725* | 35.9 | .0407 | -1.0 | .8610 | -1.0 | 2.0059* | 11.0 | 55.0 |
| % Total Necrosis | 6.2189* | 10.1 | .2298 | -7.0 | 2.3471* | 9.0 | 1.1983 | 3.3 | 84.4 |
| % Mottled Needles | 14.0596* | 30.9 | 1.2688 | -1.7 | 4.5441* | 5.3 | .2996 | 10.6 | 76.0 |
| % Healthy Needles | 6.3088* | -5.5 | .2325 | 7.3 | .2533 | -13.7 | 3.8790* | 40.9 | 71.0 |

*F ratios significant at $p \leq .05$

TABLE 4.11. SUMMARY OF F RATIOS AND % VARIANCE COMPONENTS FOR THE INDICATED LEVELS FOR ALL VARIABLES FROM THE 1975 COLLECTIONS, UPPER CROWN POSITIONS.

| | Level 3 | | Level 2 | | Level 1 | | Level 0 |
|---|---|---|---|---|---|---|---|
| | Between Plots | | Between Tree Ages Within Plots | | Between Years Foliage Within Tree Ages | | Within Years Foliage |
| Variable | F Ratio | Percent Variance Component | F Ratio | Percent Variance Component | F Ratio | Percent Variance Component | Percent Variance Component |
| Fluoride (ppm) | 9.1449* | 27.5 | 1.9399 | 3.4 | .7511 | -3.6 | 72.6 |
| Sulfur (ppm) | 3.8730 | 12.9 | 7.6885* | 10.2 | .2550 | -13.4 | 90.2 |
| Area (mm$^2$) | 5.6601* | 8.6 | 1.3488 | 1.1 | .5007 | -10 | 100 |
| Needle Length (mm) | 10.4802* | 32.9 | 1.2172 | 1.2 | 1.3740 | 4.5 | 61.2 |
| % Needle Retention | 1.4999 | 3.5 | 1.5102 | 4.1 | 1.4177 | 7.1 | 85.2 |
| % Basal Necrosis | 17.2936* | 18.8 | .0862 | -14 | 3.7867* | 34 | 61.6 |
| % Basal Scale | 3.6641 | 11.1 | .2469 | -10 | 2.9838* | 28.2 | 71.2 |
| % Defoliator | 8.9789* | 33.4 | 1.9726 | 4.0 | 1.0508 | .6 | 61.7 |
| % Tip Burn | 7.8807* | 34.8 | 1.4351 | 2.9 | 1.9609* | 10 | 52.1 |
| % Total Necrosis | .6175 | -6.9 | 3.6924* | 16.0 | .9812 | -3.4 | 91.2 |
| % Mottled Needles | 6.5830* | 26.5 | 6.8488* | 9.1 | .3136 | -10.2 | 74.5 |
| % Healthy Needles | 1.5612 | 7.4 | .2442 | -10.8 | 2.8365* | 27.7 | 75.6 |

*F ratios significant at $p \leq .05$

TABLE 4.12. SUMMARY OF F RATIOS AND % VARIANCE COMPONENTS FOR THE INDICATED LEVELS FOR ALL VARIABLES FROM THE 1975 COLLECTIONS, LOWER CROWN POSITIONS.

| | Level 3 | | Level 2 | | Level 1 | | Level 0 |
|---|---|---|---|---|---|---|---|
| | Between Plots | | Between Tree Ages Within Plots | | Between Years Foliage Within Tree Ages | | Within Years Foliage |
| Variable | F Ratio | Percent Variance Component | F Ratio | Percent Variance Component | F Ratio | Percent Variance Component | Percent Variance Component |
| Fluoride (ppm) | 6.4123* | 14 | .6068 | -2.9 | 1.3845 | 6.3 | 82.5 |
| Sulfur (ppm) | 4.2540 | 16.3 | 2.3751 | 6.3 | .8698 | -2 | 79.3 |
| Area (mm²) | 3.1453 | 9.2 | 6.9123* | 10.5 | .2856 | -13.3 | 93.6 |
| Needle Length (mm) | 13.7680* | 32.8 | 1.2750 | 1.1 | .9044 | -1.2 | 67.3 |
| % Needle Retention | 4.6608 | 11.3 | .2831 | -8.7 | 2.4079* | 21.4 | 76.0 |
| % Basal Necrosis | 4.4609 | 12.8 | .3060 | -9.2 | 2.8310* | 25.8 | 70.5 |
| % Basal Scale | 2.3684 | 13.2 | .9208 | -1.0 | 3.3777* | 27.9 | 59.8 |
| % Defoliator | 11.9581* | 36.9 | 1.1603 | .9 | 1.5131 | 5.7 | 56.3 |
| % Tip Burn | 20.1396* | 35.0 | .4319 | -4.4 | 2.0409* | 11.9 | 57.4 |
| % Total Necrosis | 1.0794 | .54 | 1.7427 | 5.7 | 1.3306 | 5.8 | 87.8 |
| % Mottled Needles | 29.0267* | 27.1 | 1.3147 | .49 | .2819 | -12.1 | 84.4 |
| % Healthy Needles | .4061 | 9.0 | .2599 | -14.8 | 5.2829* | 48.8 | 56.9 |

*F ratios significant at $p \leqq .05$

TABLE 4.13. SUMMARY OF ANALYSES OF VARIANCE FOR 1975 COLLECTION.

| | Between the Plots | | | Between the Tree Ages Within the Plots | | | Between the Years of Foliage | | |
|---|---|---|---|---|---|---|---|---|---|
| Variable | (Level 4) Both Crown Positions | (Level 3) Upper Crown Position | (Level 3) Lower Crown Position | (Level 3) Both†† Crown Positions | (Level 2) Upper††† Crown Position | (Level 2) Lower††† Crown Position | (Level 2) Both Crown Positions | (Level 1) Upper Crown Position | (Level 1) Lower Crown Position |
| Fluoride (ppm) | * | * | * | | | | | | |
| Sulfur (ppm) | * | | | * | * | | | | |
| Area ($mm^2$)† | | * | | * | | * | | | |
| Needle Length (mm) | * | * | * | | | | | | |
| % Needle Retention | * | | | | | | * | | * |
| % Basal Necrosis | * | * | | | | | * | * | * |
| % Basal Scale | * | | | | | | * | * | * |
| % Defoliator | * | * | * | | | | | | |
| % Tip Burn | * | * | * | | | | * | * | * |
| % Total Necrosis | * | | | * | * | | | | |
| % Mottled Needles | * | * | * | * | * | | | | |
| % Healthy Needles | * | | | | | | * | * | * |

† This variable showed significant differences between the Crown Positions
†† This level is actually Between the Tree Ages Within the Crown Positions
††† This level is Between the Tree Ages Within the Plots
* Significant treatment effect at $p \leq .05$

## Fluoride

The only significant effect for fluoride in the three-or four-level nested ANOVA was between the plots for all crown positions as shown in Table 4.13. No significant treatment effects for fluoride were detected between tree ages or between years of foliage. T-tests were performed between the categories of tree age and crown positions for fluoride data for foliage from all plots to confirm this, and the t-statistics for plot SE-2 are presented in Figure 4.1 (plot SE-2 was representative of all five plots for fluoride). Figure 4.2 was prepared to show the mean values for fluoride in 1974 foliage for all upper crown samples from all five plots, and this figure illustrates the difference in mean values between the plots. The mean fluoride values shown in Figure 4.2 between plots SE-4 and those of E-1, SE-2, and S-5 were significantly different at $p \leqq .05$.

## Sulfur

Significant treatment effects between the plots and between the tree ages for sulfur levels were detected (Table 4.10, Levels 2 and 4) by ANOVA employing both crown positions and by ANOVA employing the upper crown position (Table 4.11, Level 2), which suggests greater variation between upper crowns of the two tree ages than between the lower crown positions.

T-tests of sulfur levels between the individual years of foliage from upper crowns of both older trees and younger trees, tested separately, revealed no significant differences ($p \leqq .05$). Thus, the mean values of sulfur concentrations for upper crown younger trees and upper crown older trees were recomputed by pooling the measurements for the individual years. These means for upper crown younger trees and upper crown older trees were then compared utilizing the t-test. Plot SE-4 was the only plot which showed significant differences for sulfur between the tree ages at $p \leqq .05$, and the basic statistics are presented in Table 4.14. The means and 95% confidence intervals for sulfur levels between upper crown, younger and older trees are shown in Figure 4.3 for plot S-5.

## Cross-Sectional Area

For needle fascicular cross-sectional area, ANOVA indicated significant treatment effects (Table 4.13) between the plots for the upper crown position. Also, significant effects were found between the tree ages for the lower and for the combined crown positions (see Table 4.13) and between the crown positions for both crown analyses. It should be expected that the variability of cross-sectional area of fascicles from lower crown foliage within the plots will be greater than between the plots. Figure 4.4 illustrates the mean values and 95% confidence intervals for fascicular cross-sectional areas from the upper and lower crown positions for plot SE-4. As is depicted in Figure 4.4, the difference between the crown positions was most apparent for the younger trees and fascicular cross-sectional area was generally smaller for the lower crown position.

| | | UO | LY | LO |
|---|---|---|---|---|
| | UY | .416 | .162 | 1.068 |
| 1974 | UO | | .461 | 1.473 |
| | LY | | | .913 |

| | ZO | LZ |
|---|---|---|
| XY | .625 | |
| UZ | | 1.163 |

| | | UO | LY | LO |
|---|---|---|---|---|
| | UY | .035 | .069 | .382 |
| 1973 | UO | | .063 | .654 |
| | LY | | | .698 |

| | ZO | LZ |
|---|---|---|
| ZY | .388 | |
| UZ | | 328 |

| | | UO | LY | LO |
|---|---|---|---|---|
| | UY | .100 | 1.343 | .149 |
| 1972 | UO | | 1.921 | .331 |
| | LY | | | 1.572 |

| | ZO | LZ |
|---|---|---|
| ZY | 1.023 | |
| UZ | | 1.279 |

*t.05 = 2.306 for UY, UO, LY, LO Comparisons
t.05 = 2.101 for ZY, ZO, UZ, LZ Comparisons

**UY = Upper Crown, Younger Trees
UO = Upper Crown, Older Trees
LY = Lower Crown, Younger Trees
LO = Lower Crown, Older Trees

ZY = All Crowns, Younger Trees
ZO = All Crowns, Older Trees
UZ = Upper Crowns, All Tree Ages
LZ = Lower Crown, All Tree Ages

Figure 4.1. Results of t-tests* between the categories** of tree age and crown position for 1974, 1973, and 1972 years of foliage from plot SE-2.

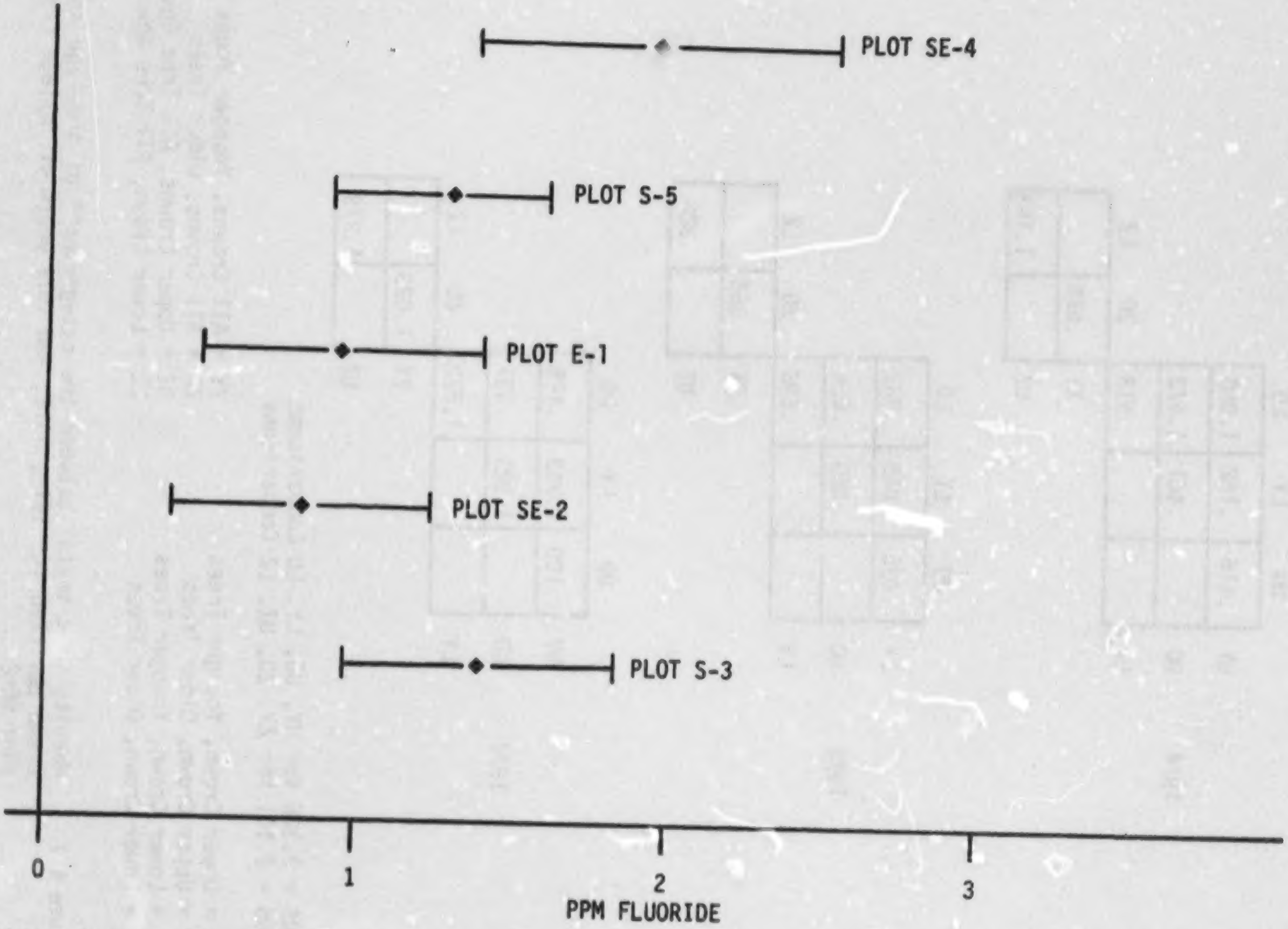

Figure 4.2. Mean values and 95% confidence interval widths for fluoride in 1974 foliage, upper crown, all tree ages.

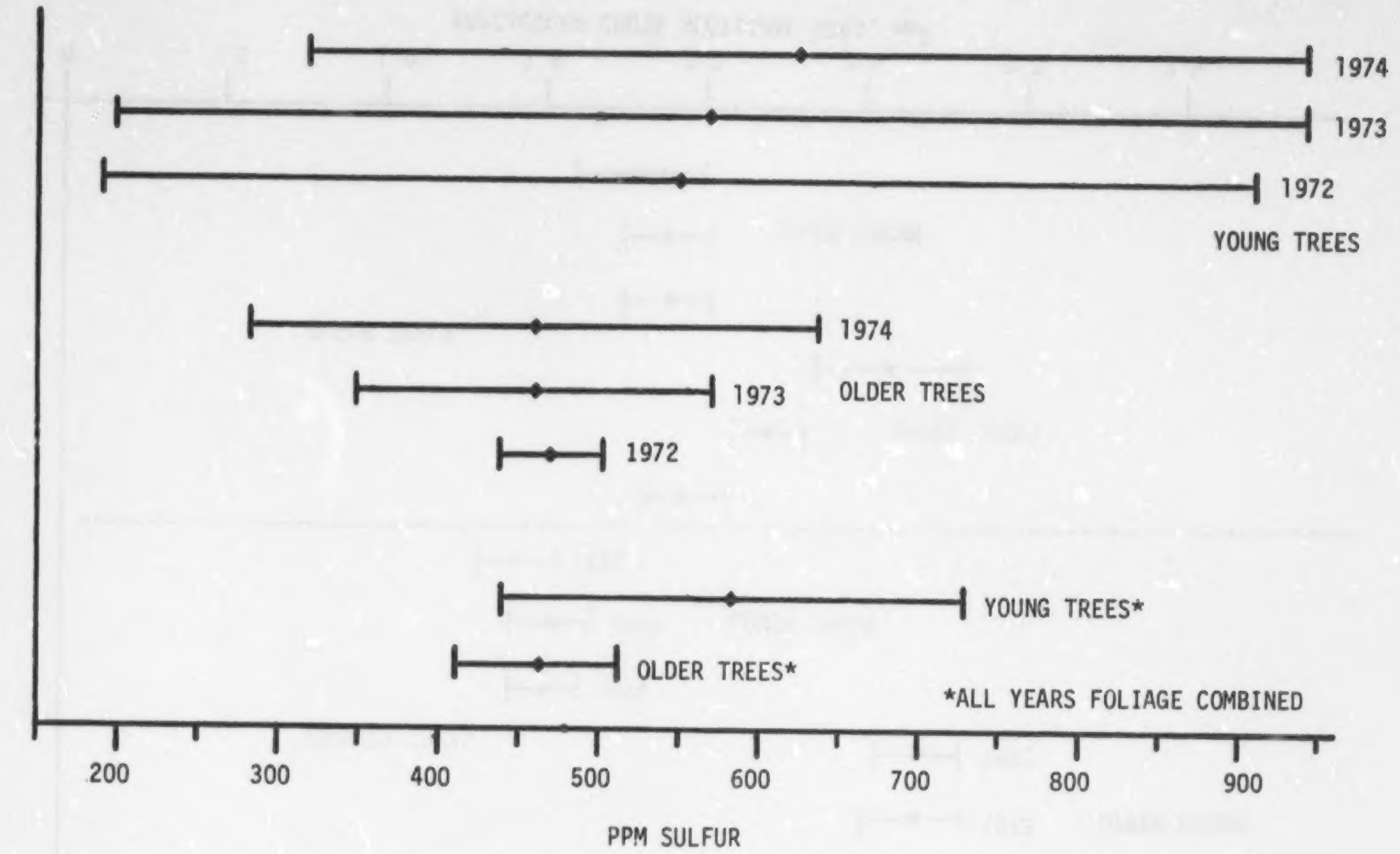

Figure 4.3. Variation in total sulfur concentration in upper crown younger and older trees for plot S-5.

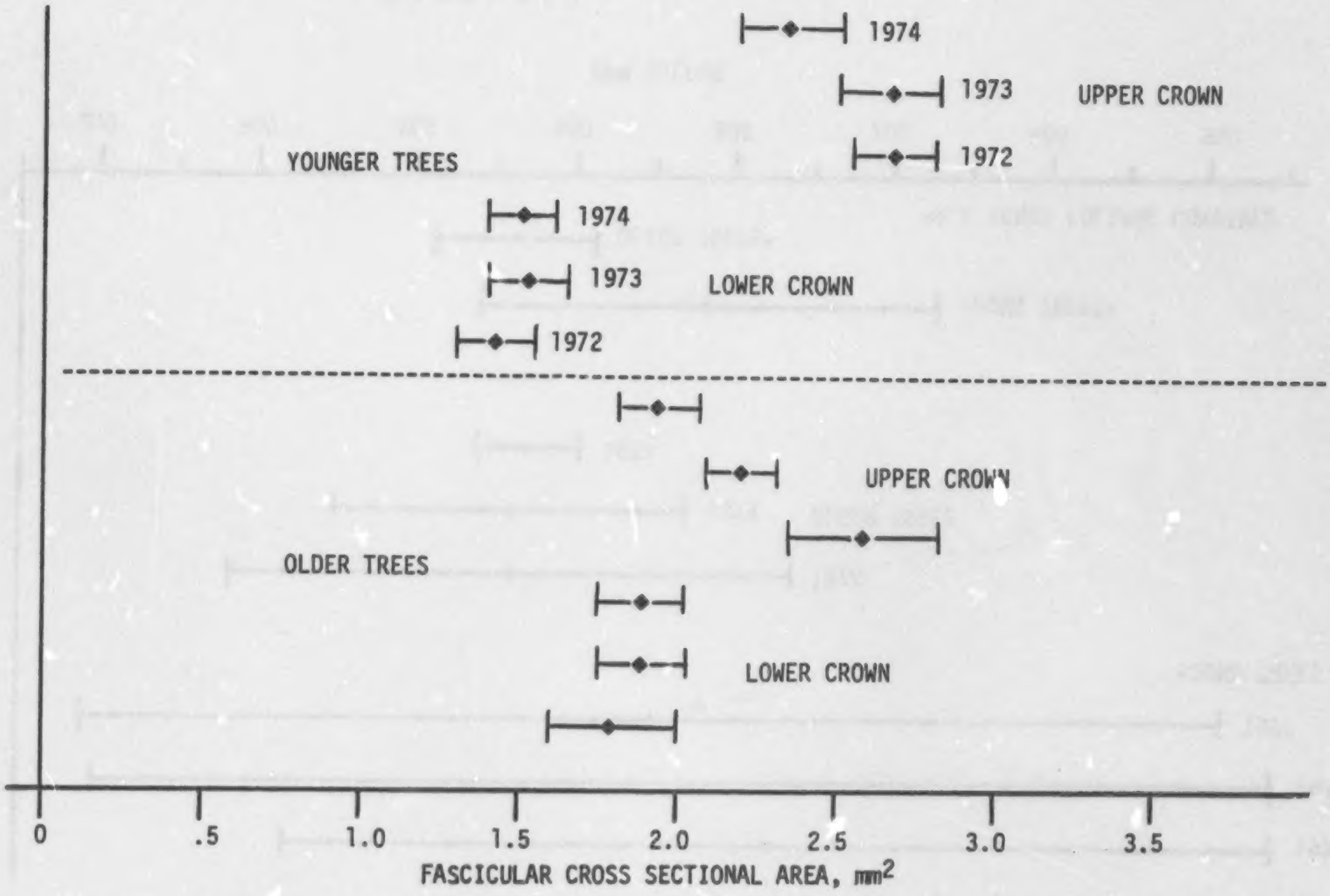

Figure 4.4. Mean values and 95% confidence intervals for fascicular cross-sectional area, upper and lower crown positions, younger and older trees, plot SE-4.

TABLE 4.14. BASIC STATISTICS FOR SULFUR CONCENTRATIONS IN UPPER CROWN YOUNGER TREES AND UPPER CROWN OLDER TREES, ALL YEARS FOLIAGE COMBINED, PLOT SE-4*

| Statistic | Upper Crown, Younger | Upper Crown, Older |
|---|---|---|
| N | 15 | 15 |
| Maximum Value | 550 | 500 |
| Minimum Value | 300 | 250 |
| Mean | 420 | 348 |
| Variance | 5285.714 | 6291.666 |
| Standard Deviation | 72.03 | 79.320 |
| Standard Error | 18.772 | 20.480 |
| 95% Confidence Limit | 40.266 | 43.930 |
| Median | 450 | 350 |
| Standard Error | 23.5 | 25.666 |
| Coefficient of Variation | 17.31 | 22.771 |
| Standard Error | 3.25 | 4.36 |
| Sampling Precision** | 9.587 | 12.612 |

*t-statistic @ $p \leq .05 = 2.048$
t-statistic between the mean values = 2.580

**Defined as $\left[\frac{(t_{.05} S_{\bar{x}})}{\bar{x}}\right] 100$

## Needle Length

Significant treatment effects were found for needle length between plots but not within plots (Table 4.13). The mean values for needle length for each year's foliage for upper and lower crown, younger and older trees for each plot were compared by t-tests and the results are summarized in Table 4.15. As is depicted in this table, needle length across the plots was generally shorter on the lower crowns than on the upper crowns of younger trees, while needle length of older trees of both upper and lower crowns was generally the same. The upper crowns of younger trees tended to have longer needles than the upper crowns of older trees.

## Needle Retention

ANOVA showed significant treatment effects for needle retention between the plots (Table 4.13) and between the years of foliage for both crown positions and the lower crown position but not for the upper crown position. The mean values and 95% confidence intervals for percent needle retention for upper and lower crown positions for plot E-1 are shown in Figure 4.5 and these values are generally representative of the other plots. The obvious differences between 1974 percent needle retention and older foliage from the lower crown as depicted in Figure 4.6 is representative of the patterns at the other plots with the exception of plot S-3 where the percent needle retention on the lower crowns was

TABLE 4.15. RESULTS OF T-TESTS BETWEEN MEAN VALUES FOR NEEDLE LENGTH FOR TREE AGES AND CROWN POSITIONS FOR ALL PLOTS.

| Plot | Year of Foliage Origin | Mean Values for Needle Length | | | |
|---|---|---|---|---|---|
| | | UY* vs. | UO* | LY* vs. | LO* |
| SE-4 | 1974 | 110.7 | 131.2† | 126.6 | 135.9† |
| | 1973 | 127.6 | 124.6 | 118.1 | 125.0† |
| | 1972 | 117.3 | 111.5† | 111.6 | 111.7 |
| S-5 | 1974 | 128.3 | 115.5† | 120.0 | 122.5 |
| | 1973 | 150.2 | 141.5† | 127.5 | 125.4 |
| | 1972 | 146.1 | 136.7† | 122.5 | 110.3† |
| E-1 | 1974 | 151.0 | 152.9 | 152.7 | 146.5† |
| | 1973 | 160.8 | 153.3† | 144.1 | 151.1† |
| | 1972 | 160.2 | 145.9† | 145.1 | 140.4 |
| SE-2 | 1974 | 134.1 | 119.9† | 127.3 | 122.3† |
| | 1973 | 145.7 | 137.5† | 125.3 | 138.1† |
| | 1972 | 130.5 | 135.2† | 121.8 | 128.2† |
| S-3 | 1974 | 130.1 | 119.0† | 127.8 | 113.7† |
| | 1973 | 138.0 | 123.5† | 126.8 | 114.6† |
| | 1972 | 120.3 | 111.4† | 116.9 | 110.4† |

| Plot | Year of Foliage Origin | Mean Values for Needle Length | | | |
|---|---|---|---|---|---|
| | | UY* vs. | LY* | UO* vs. | LO* |
| SE-4 | 1974 | 110.7 | 126.6† | 131.2 | 135.9 |
| | 1973 | 127.6 | 118.1† | 124.6 | 125.0 |
| | 1972 | 117.3 | 111.6† | 111.5 | 111.7 |
| S-5 | 1974 | 128.3 | 120.0† | 115.5 | 122.5† |
| | 1973 | 150.2 | 127.5† | 141.5 | 125.4† |
| | 1972 | 146.1 | 122.5† | 136.7 | 110.3† |
| E-1 | 1974 | 151.0 | 152.7 | 152.9 | 146.5 |
| | 1973 | 160.8 | 144.1† | 153.3 | 151.0 |
| | 1972 | 160.2 | 145.1† | 145.9 | 140.4 |
| SE-2 | 1974 | 134.1 | 127.3† | 119.9 | 122.3 |
| | 1973 | 145.6 | 125.3† | 137.5 | 138.1 |
| | 1972 | 130.5 | 121.8† | 135.2 | 128.2† |
| S-3 | 1974 | 130.1 | 127.8† | 119.0 | 113.7† |
| | 1973 | 138.0 | 126.8† | 123.5 | 114.6† |
| | 1972 | 120.3 | 116.9 | 111.5 | 110.4 |

*UY = Upper Crown, Younger Trees LY = Lower Crown, Younger Trees
UO = Upper Crown, Older Trees LO = Lower Crown, Older Trees

†Mean values are significantly different @ $p \leq .05$

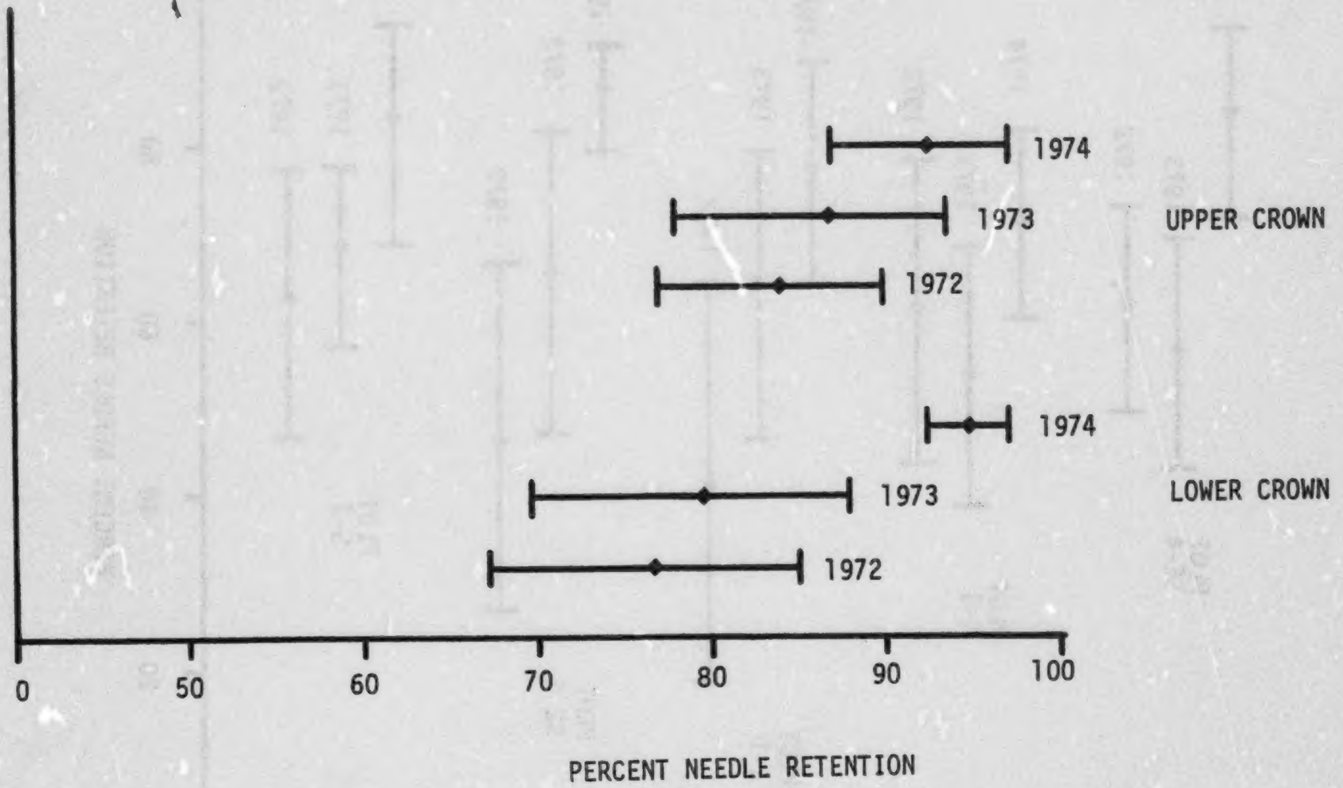

Figure 4.5. Mean values and 95% confidence intervals for percent needle retention for upper and lower crown positions, plot E-1.

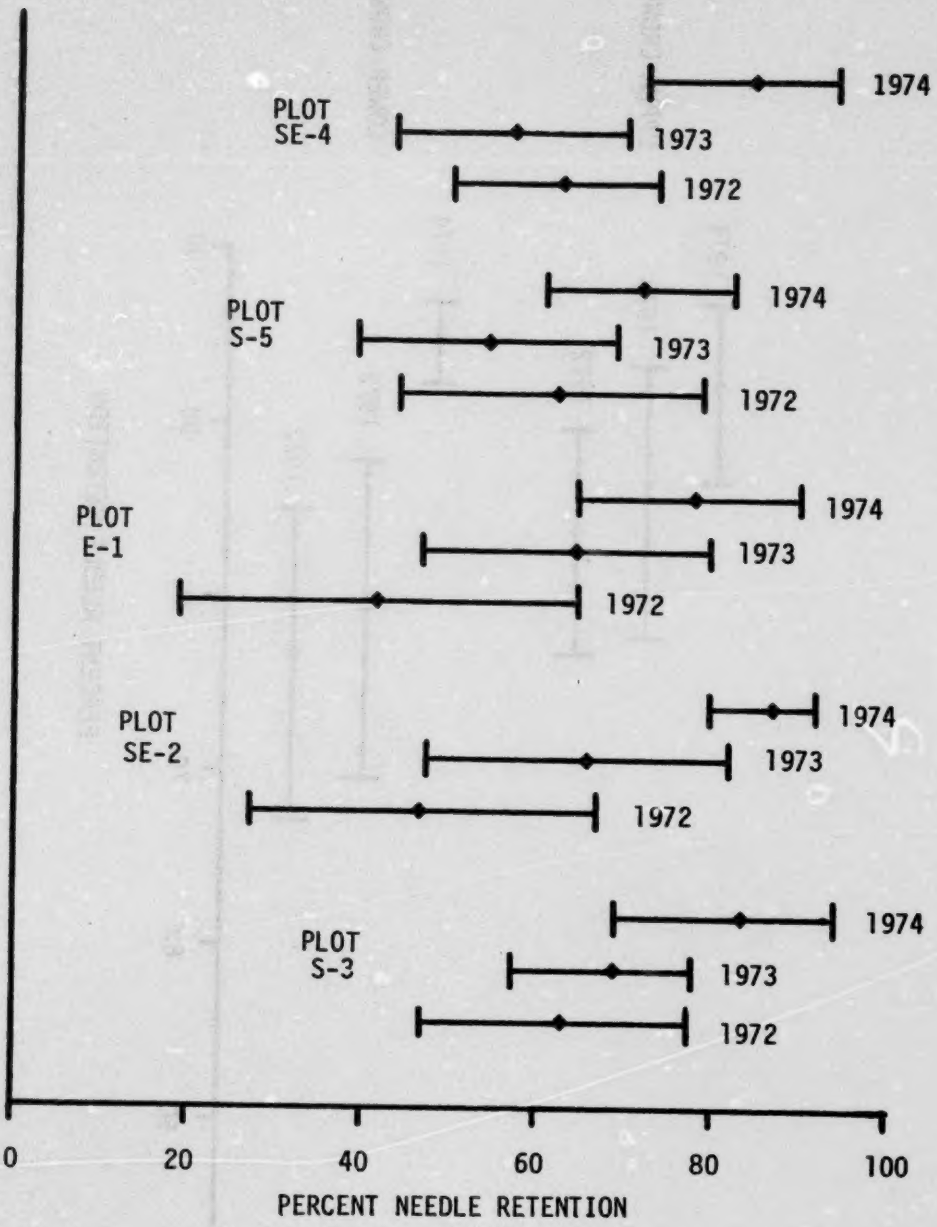

Figure 4.6. Mean values and 95% confidence intervals for percent needle retention for upper crown positions for all plots.

90 percent for all internodes. At all plots, mean percent needle retention decreases with internode age for upper crown positions, but the differences were usually significant only between the youngest internodes (i.e., 1974 foliage) and the oldest internodes (i.e., 1972 foliage) being examined.

## Basal Necrosis

As depicted in Table 4.13, significant treatment effects for basal necrosis were found between the plots and between the years of foliage for both crown positions and for the upper crown position, while significant treatment effects in the lower crown position were found only between the years of foliage. The mean values for percent basal needle necrosis and the 95% confidence intervals for upper and lower crown positions for plots E-1 and SE-2 are shown in Figure 4.7. As is depicted in this figure, the mean values for percent basal necrosis increased with internode age in general, but differences between the mean values were not always significant across the plots.

## Basal Scale

The ANOVA of basal scale showed that there were significant treatment effects in this pathology for both crown positions between the plots and between the years of foliage. For the upper and lower crown positions, significant treatment effects were indicated between the different years of foliage (see Table 4.13). No significant treatment effects were detected for basal scale due to tree age. Percent basal scale is quite variable, and the mean values are imprecisely known. On Table 4.16 are the basic statistics for percent basal scale for the three different-aged foliage from plot S-3, and these data are illustrative of the variability of the data from the other plots.

## Defoliator

For percent defoliator, the ANOVA detected significant treatment effects between the plots for both crown positions and for lower and upper crown positions, but not between the tree ages nor between the years of foliage, which indicates that the variability for this pathology was greater between the plots than within the plots (see Table 4.13). As was found for basal scale, percent defoliator was a very variable needle pathology. The mean values and 95% confidence intervals for all plots, upper and lower crown positions, are arrayed in Table 4.17.

## Tip Burn

In Table 4.13, significant treatment effects between the plots and between the different years of foliage were detected for percent tip burn by each ANOVA. No significant effects were found between the younger and older trees or between crown positions. The basic statistics for percent tip burn for the three different-aged foliage from plots S-3 and SE-2 are presented in Table 4.18.

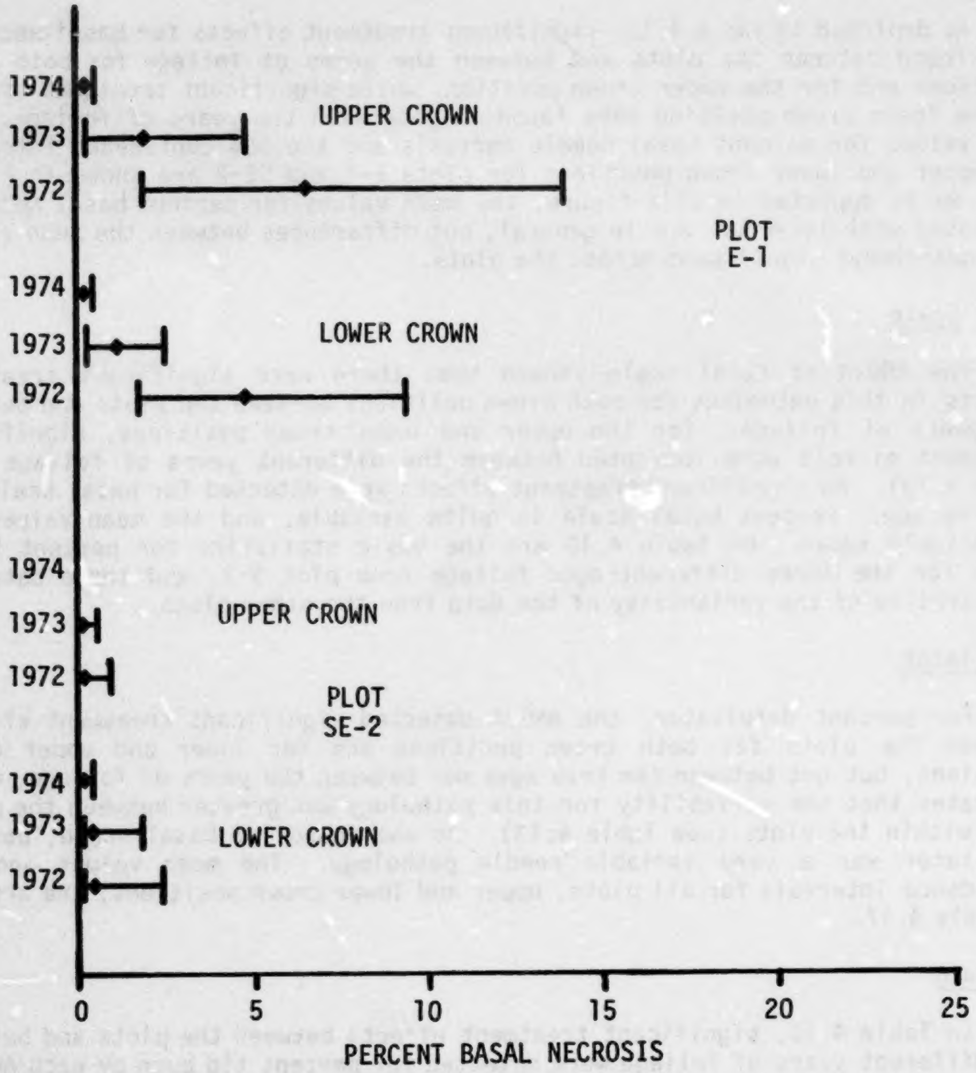

Figure 4.7. Percent basal necrosis for upper and lower crown positions, plots E-1 and SE-2, illustrating differences between internodes and between plots.

TABLE 4.16. BASIC STATISTICS FOR PERCENT BASAL SCALE FOR 1974, 1973, AND 1972 YEARS FOLIAGE FROM PLOT S-3.

| | 1974 | 1973 | 1972 |
|---|---|---|---|
| N | 20 | 20 | 20 |
| Maximum Value | 12 | 37 | 54 |
| Minimum Value | 0 | 0 | 0 |
| Mean | .69 | 8.23 | 12.52 |
| Standard Deviation | 6.00 | 10.94 | 13.72 |
| 95% Confidence Intervals | +1.05 | +5.55 | +8.29 |
| | -.57 | -4.22 | -6.42 |
| Median | .251 | 8.49 | 10 |
| Standard Error | 1.682 | 3.06 | 3.84 |
| Coefficient of Variation | 125.1 | 65.66 | 66.22 |
| Standard Error | 40.25 | 14.17 | 14.34 |

TABLE 4.17. MEAN VALUES AND 95% CONFIDENCE INTERVALS FOR PERCENT DEFOLIATOR FOR UPPER AND LOWER CROWN POSITIONS, ALL PLOTS.

| | Upper Crown | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1974 | | | 1973 | | | 1972 | | |
| Plot | $\bar{x}$ | + | - | $\bar{x}$ | + | - | $\bar{x}$ | + | - |
| SE-4 | 0 | | | .11 | .43 | .11 | .02 | .19 | .01 |
| S-5 | .44 | 1.3 | .44 | 1.44 | 2.0 | 1.15 | 1.20 | 1.62 | .94 |
| E-1 | 2.93 | 3.67 | 2.28 | 4.41 | 3.70 | 2.62 | 7.51 | 11.12 | 6.34 |
| SE-2 | 2.89 | 3.03 | 1.97 | 2.59 | 3.23 | 1.59 | 8.41 | 4.19 | 3.40 |
| S-3 | 3.41 | 5.44 | 2.93 | 5.99 | 4.14 | 3.10 | 8.18 | 10.19 | 6.53 |

| | Lower Crown | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1974 | | | 1973 | | | 1972 | | |
| Plot | $\bar{x}$ | + | - | $\bar{x}$ | + | - | $\bar{x}$ | + | - |
| SE-4 | .07 | .42 | .05 | .04 | .21 | .03 | .11 | .63 | .08 |
| S-5 | 1.89 | 2.16 | 1.35 | 2.74 | 2.64 | 1.77 | 4.10 | 3.45 | 2.43 |
| E-1 | 1.98 | 4.78 | 1.94 | 5.96 | 7.98 | 4.75 | 9.09 | 4.58 | 3.72 |
| SE-2 | 2.06 | 2.79 | 1.63 | 6.37 | 4.77 | 3.51 | 8.68 | 4.38 | 3.56 |
| S-3 | 6.22 | 6.38 | 4.24 | 7.85 | 5.43 | 4.10 | 9.08 | 6.74 | 5.01 |

TABLE 4.18. BASIC STATISTICS FOR % TIP BURN FOR THE INDICATED YEARS FOLIAGE FOR PLOTS SE-2 AND PLOTS S-3.

| Statistic | SE-2* 1974 | SE-2* 1973 | SE-2* 1972 | S-3* 1974 | S-3* 1973 | S-3* 1972 |
|---|---|---|---|---|---|---|
| N | 20 | 20 | 20 | 20 | 20 | 20 |
| Maximum Value | 12 | 52 | 73 | 4 | 6 | 2 |
| Minimum Value | 0 | 0 | 0 | 0 | 0 | 0 |
| Mean | 2.01 | 10.5 | 23.2 | .08 | .03 | .07 |
| Variance | 50.9 | 200 | 335 | 12.7 | 11.21 | 7.87 |
| Standard Deviation | 7.13 | 14.1 | 18.3 | 3.56 | 3.35 | 2.8 |
| 95% Confidence Interval | +1.95<br>-1.30 | +8.0<br>-5.98 | +13.64<br>-11.26 | +.25<br>-.08 | +.017<br>-.020 | +.17<br>-.07 |
| Median | 1.45 | 5.0 | 25.9 | 0 | 0 | 0 |
| 95% Confidence Interval | +2.26<br>-1.22 | +8.0<br>-4.35 | +17.7<br>-14.4 | +.13<br>-.13 | +.11<br>-.11 | +.08<br>-.08 |
| Coefficient of Variation | 87.59 | 74.6 | 63.5 | 212.8 | 337.0 | 180.4 |
| Standard Error | 22.0 | 17.1 | 13.5 | 106.7 | 259.6 | 78.2 |
| Sampling Precision** | 81.2 | 66.6 | 53.6 | 199 | 315.3 | 168.8 |

*The data arrayed herein are for all categories of tree age and crown position combined.

**Defined as $\left[\frac{(t_{.05} S_{\bar{x}})}{\bar{x}}\right] 100$

### Total Needle Necrosis

ANOVA showed significant treatment effects between the plots and between the tree ages for both crown positions (Table 4.13). Significant effects were detected for the different-aged trees for upper crown but not lower crown. No significant effects of percent total needle necrosis were found between the crown positions or between the different years of foliage. The mean values and the 95% confidence intervals for percent needle necrosis for SE-2 are presented in Figure 4.8.

### Needle Mottling

As shown in Table 4.13, the ANOVA detected significant treatment effects in percent mottled needles between the plots for the three categories of crown positions, and in the two tree ages for both crown positions and the upper crown position. No differences were detected due to years of foliage origin. The mean values and 95% confidence intervals for the foliage of upper crowns of younger and older trees from plots SE-2 and S-3 are depicted in Figure 4.9.

### Healthy Needles

Significant treatment effects were detected between plots for the ANOVA employing both crown positions and for all three combinations of crown positions between the years of foliage (see Table 4.13). Mean values for percent healthy needles and the 95% confidence intervals for the upper crown positions for all five ponderosa sites are depicted in Figure 4.10.

## 1975-1976 DATA ANALYSES COMPARISON

The data from the 1976 collections were treated by a three-level nested ANOVA, and the results are summarized as Table 4.19. In addition, the data for foliage from the upper crown positions between collections for both years were treated by four-level nested ANOVA, and the results are shown in Table 4.20. Tables 4.11, 4.19, and 4.20 are summarized in Table 4.21. In this table the four-level nested ANOVA between the 1975 and 1976 collections is designated ANOVA K, the three-level nested ANOVA for upper crown foliage from the 1975 collections is designated ANOVA B, and the three-level nested ANOVA for the 1976 collection is designated ANOVA J. For each variable in Table 4.21, a significant treatment effect ($p \leq .05$) for a particular level, for each ANOVA (K, B, or J, as above), is denoted by an asterisk (*). Then, for ppm fluoride (refer to Table 4.21) significant treatment effects were detected: (1) Between the two years' collections (Level 4, ANOVA K), and (2) between the plots (Level 3, ANOVAs K and B).

Significant treatment effects between the 1975 and 1976 collections (Table 4.21, ANOVA K) were detected only for: (1) ppm fluoride, (2) percent total necrosis, and (3) percent healthy needles. Significant treatment effects only between the plots were indicated for area ($mm^2$), needle length (mm), and percent defoliator by each ANOVA. For percent basal scale, significant

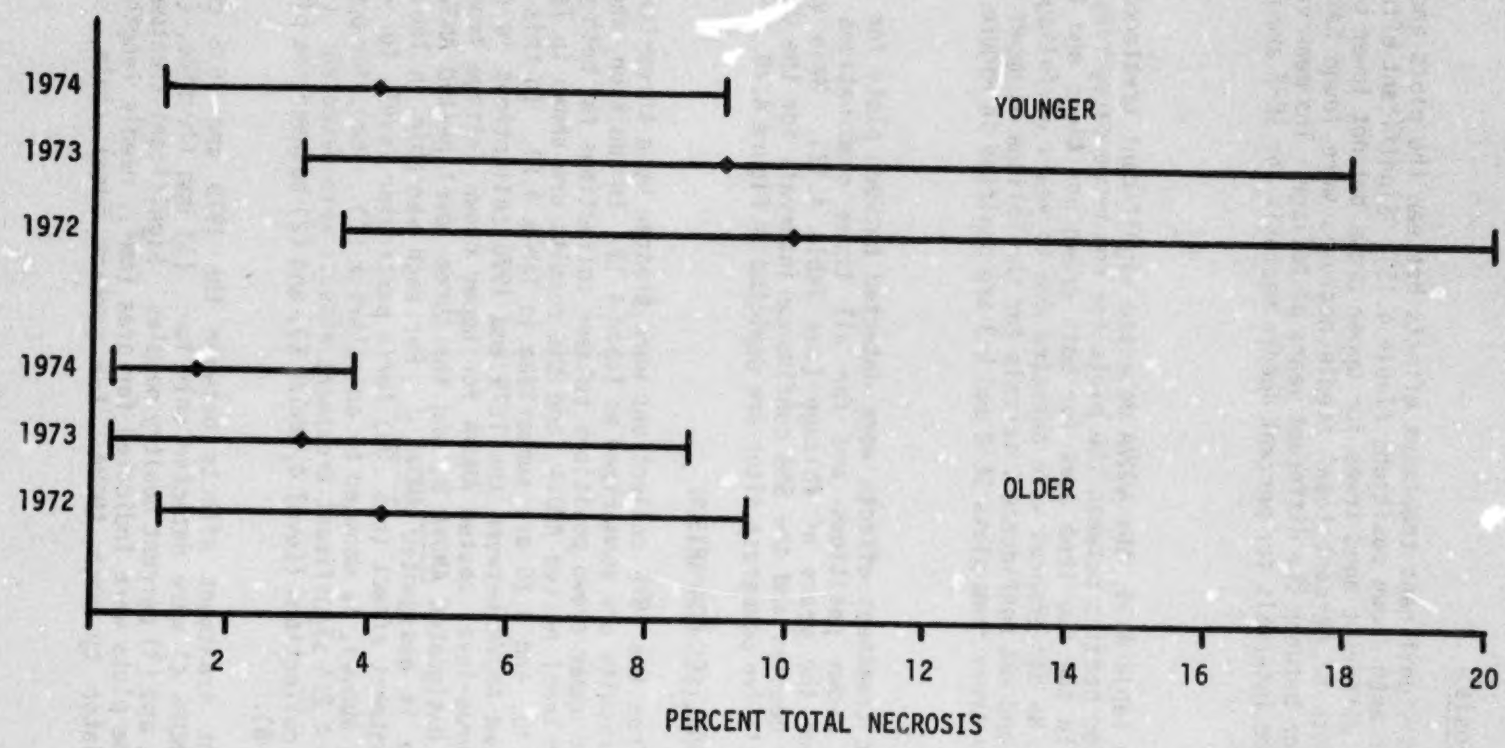

Figure 4.8. Mean values and 95% confidence intervals for percent total necrosis illustrating differences in mean values for upper crown, younger and older trees at plot SE-2.

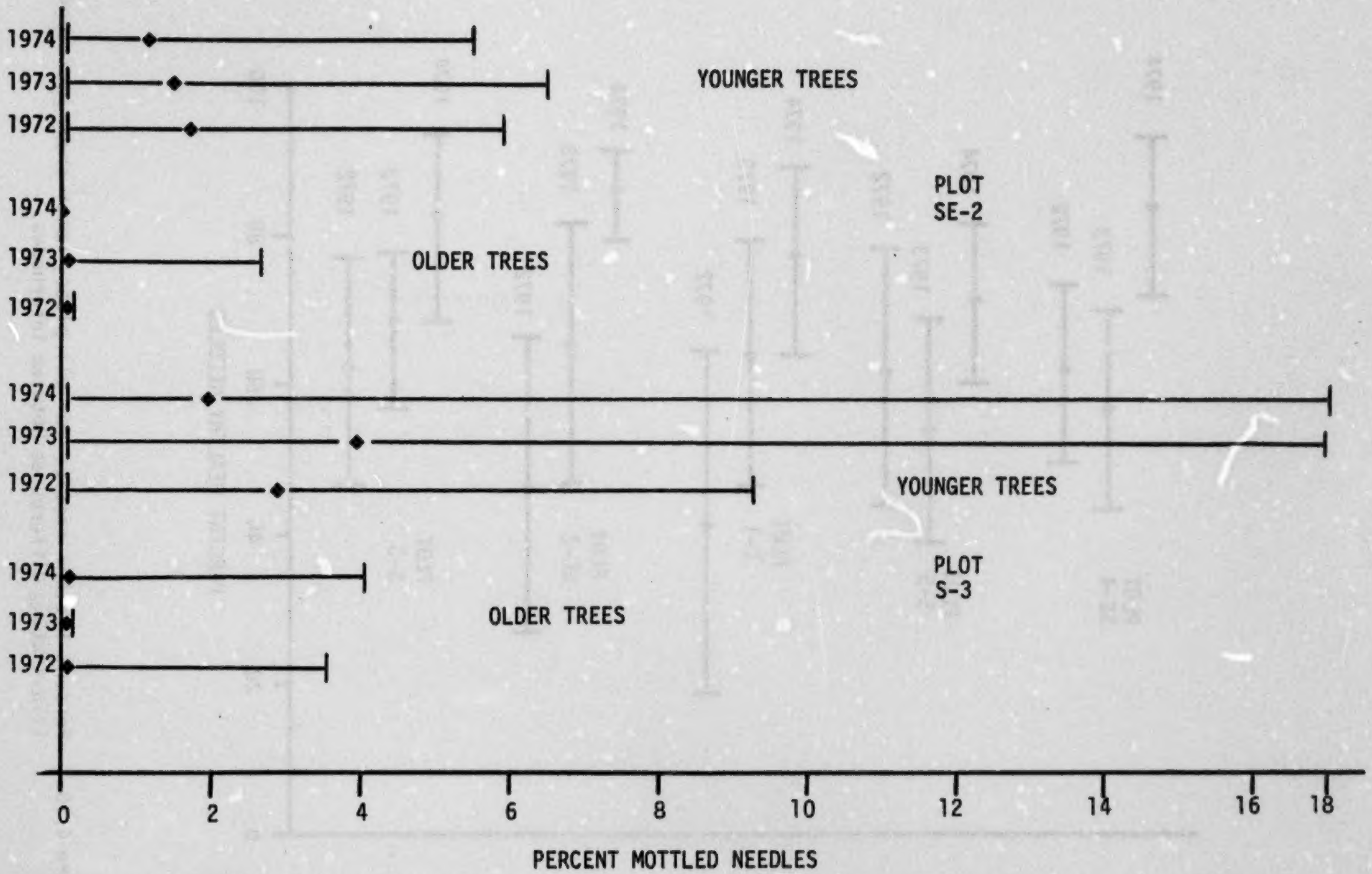

Figure 4.9. Mean values and 95% confidence intervals for percent mottled needles illustrating differences in mean values for upper crown, younger and older trees for plots SE-2 and S-3.

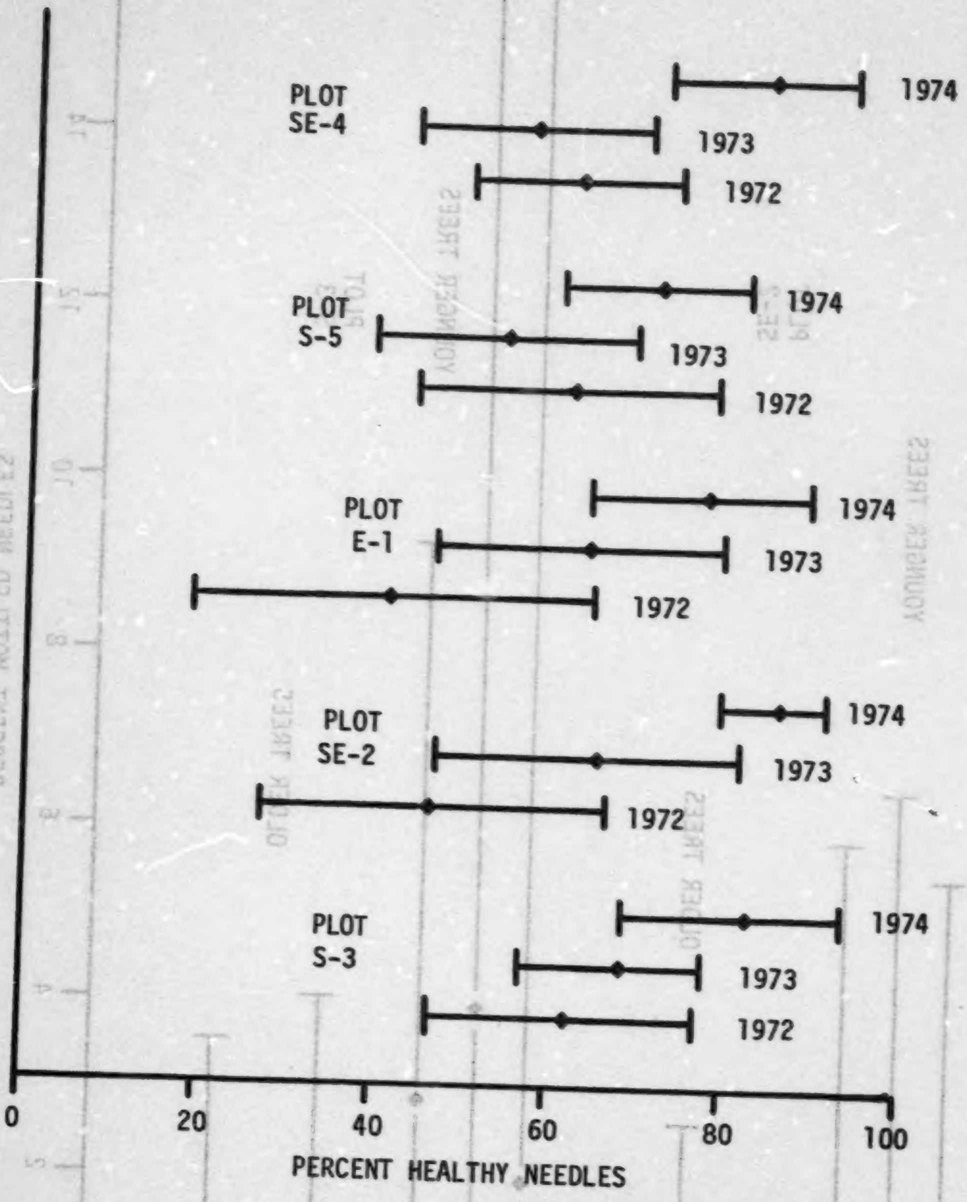

Figure 4.10. Percent healthy needles for all plots, upper crown positions, illustrating differences between internodes.

TABLE 4.19. SUMMARY OF F RATIOS AND % VARIANCE COMPONENTS FOR THE INDICATED LEVELS FOR ALL VARIABLES FROM THE 1976 COLLECTIONS, UPPER CROWN POSITIONS.

| | Level 3 Between Plots | | Level 2 Between Tree Ages Within Plots | | Level 1 Between Years Foliage Within Tree Ages | | Level 0 Within Years Foliage |
|---|---|---|---|---|---|---|---|
| Variable | F Ratio | Percent Variance Component | F Ratio | Percent Variance Component | F Ratio | Percent Variance Component | Percent Variance Component |
| Fluoride (ppm) | 2.0250 | 3.12 | 1.5327 | 2.83 | .8190 | -3.53 | 97.5 |
| Sulfur (ppm) | 10.5296* | 42.31 | 4.3590* | 7.16 | .5659 | -4.91 | 55.43 |
| Area (mm$^2$) | 6.6297* | 18.43 | 1.2654 | 1.39 | .9797 | - .32 | 80.49 |
| Needle Length (mm) | 10.2867* | 31.05 | 1.7671 | 2.97 | .8582 | -1.92 | 67.89 |
| % Needle Retention | 1.6463 | 7.75 | .3445 | -9.29 | 2.8835* | 27.78 | 73.75 |
| % Basal Necrosis | 14.6891* | 29.31 | .6162 | -2.53 | 1.4812 | 6.42 | 66.79 |
| % Basal Scale | 1.980 | 13.52 | .3589 | -14.82 | 8.6873* | 61.37 | 39.92 |
| % Defoliator | 6.8075* | 22.31 | 2.0275 | 4.11 | .7805 | -3.37 | 76.94 |
| % Tip Burn | 7.5404* | 24.48 | .3695 | -9.11 | 4.2017* | 33.03 | 51.59 |
| % Total Necrosis | 2.7747 | 12.97 | .4701 | -7.93 | 3.5910* | 32.41 | 62.54 |
| % Mottled Needles | 67.2327* | 27.41 | .0899 | -7.45 | 1.7716* | 10.70 | 69.34 |
| % Healthy Needles | 11.9031* | 22.92 | .0803 | -20.41 | 8.6191* | 58.86 | 36.62 |

*Indicates F ratio signficant @ $p \leq .05$

TABLE 4.20. SUMMARY OF F RATIOS AND % VARIANCE COMPONENTS FOR THE INDICATED LEVELS FOR ALL VARIABLES FROM THE 1975 AND 1976 COLLECTIONS, UPPER CROWN POSITIONS.

| | Level 4 | | Level 3 | | Level 2 | | Level 1 | | Level 0 |
|---|---|---|---|---|---|---|---|---|---|
| | Between Collections | | Between Plots Within Collections | | Between Tree Ages Within Plots | | Between Years Foliage Within Tree Ages | | Within Years Foliage |
| Variable | F Ratio | Percent Variance Component | F Ratio | Percent Variance Component | F Ratio | Percent Variance Component | F Ratio | Percent Variance Component | Percent Variance Component |
| Fluoride (ppm) | 7.5576* | 21.45 | 5.9252* | 13.65 | 1.7318 | 2.49 | .7843 | -2.81 | 65.20 |
| Sulfur (ppm) | 2.2318 | 6.06 | 7.2439* | 27.74 | 5.5503* | 7.99 | .3939 | -8.12 | 66.32 |
| Area (mm$^2$) | 1.2522 | -.80 | 6.2892* | 14.20 | 1.2935 | 1.30 | .7410 | -4.66 | 89.95 |
| Needle Length (mm) | .0479 | -11.99 | 10.3693* | 35.68 | 1.4814 | 2.49 | 1.0661 | .96 | 72.85 |
| % Needle Retention | .8059 | -1.28 | 1.5374 | 5.55 | .8097 | -2.09 | 2.0411* | 16.85 | 80.96 |
| % Basal Necrosis | 2.7653 | 3.57 | 15.1392* | 24.34 | .2987 | -6.87 | 2.3314* | 16.58 | 62.28 |
| % Basal Scale | 2.7453 | -1.65 | 1.5825 | 13.01 | .3352 | -13.78 | 6.1854* | 52.14 | 50.28 |
| % Defoliator | .6829 | -5.85 | 8.1998* | 31.03 | 1.9918 | 4.41 | .9365 | -.90 | 71.31 |
| % Tip Burn | .1085 | -10.38 | 7.7407* | 32.04 | .6565 | -4.21 | 3.2129* | 25.32 | 57.22 |
| % Total Necrosis | 5.3700* | 6.97 | 1.4337 | 5.68 | 1.0276 | .30 | 2.4593* | 19.66 | 67.36 |
| % Mottled Needles | 4.1453 | 11.19 | 19.5443* | 24.19 | .4014 | -3.63 | 1.4591* | 5.73 | 62.50 |
| % Healthy Needles | 11.2915* | 10.21 | 6.4417* | 15.52 | .1244 | -15.19 | 5.5676* | 42.70 | 46.75 |

*Indicates F ratio significant at $p \leq .05$

TABLE 4.21. SUMMARY OF RESULTS OF THREE-LEVEL NESTED ANALYSIS OF VARIANCE FOR UPPER CROWN FOLIAGE FROM THE 1975 COLLECTIONS (ANOVA B), THE 1976 COLLECTIONS (ANOVA J), AND THE FOUR-LEVEL NESTED ANALYSIS OF VARIANCE BETWEEN THE 1975 AND 1976 COLLECTIONS (ANOVA K).

| Variable | Level 4 | Level 3 | | | Level 2 | | | Level 1 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Between Collections | Between the Plots | | | Between the Tree Ages Within the Plots | | | Between the Years of Foliage Within the Tree Ages | | |
| | ANOVA | ANOVA | | | ANOVA | | | ANOVA | | |
| | K | K | B | J | K | B | J | K | B | J |
| Fluoride (ppm) | * | * | * | | | | | | | |
| Sulfur (ppm) | | * | | * | * | * | * | | | |
| Area (mm$^2$) | | * | * | * | | | | | | |
| Needle Length (mm) | | * | * | * | | | | | | |
| % Needle Retention | | | | | | | | * | | * |
| % Basal Necrosis | | * | * | * | | | | * | * | |
| % Basal Scale | | | | | | | | * | * | * |
| % Defoliator | | * | * | * | | | | | | |
| % Tip Burn | | * | * | * | | | | * | * | * |
| % Total Necrosis | * | | | | | * | | * | | * |
| % Mottled Needles | | * | * | * | | * | | * | | * |
| % Healthy Needles | * | | | * | | | | * | * | * |

* Indicates F ratio significant at $p \leq .05$

treatment effects only between the years of foliage were detected by each ANOVA, while for percent tip burn, significant treatment effects between the plots and between the years of foliage were noted by each ANOVA (Table 4.21).

Since no significant treatment effects for tree ages or age of foliage origin were detected, values for all tree ages and years of foliage were combined, and the resultant mean values and 95% confidence intervals for fluoride from upper crown positions from all plots, both collections, are shown in Figure 4.11. Whereas in 1975 the plots were significantly different in fluoride content, in general, in 1976, with the exception of plot SE-2, the plots were not significantly different.

For ppm sulfur, significant treatment effects between the plots were detected by ANOVAs K and J, but not for 1975 data alone (ANOVA B). ANOVAs B, K, and J detected significant differences for sulfur between the tree ages. These results indicate that changes in sulfur concentrations in 1976 were sufficient to cause the plots themselves to be different, but the changes were not such that the two year's collections would be different. The only statistically significant difference between older and younger trees in 1976 was at plot S-5. The basic statistics for sulfur concentrations in upper crown, younger and older trees for plot S-5 are arrayed in Table 4.22.

The mean sulfur values for younger and older trees for all plots are arrayed in matrix form as Table 4.23. For younger trees, plot SE-4 was significantly lower than all other plots, while S-5 had significantly greater concentrations than all other plots. Although plot E-1 had more sulfur than plot SE-4, it was lower than plots SE-2, S-3, and S-5. There were no significant differences between sulfur concentrations between plots S-3 and SE-2. For older trees (Table 4.23), plot SE-4 was again significantly lower in sulfur than all other plots. In addition to plot SE-4, plot S-5 had higher levels in foliage than plot E-1. No other significant differences could be shown for older trees between plots.

The mean values and 95% confidence intervals for sulfur in upper crown, trees for 1975 and 1976 collections for each plot are shown in Figure 4.12. The increases shown for plots S-5, SE-2, and S-3 in 1976 over 1975 are significant at $p \leqq .05$.

For percent needle retention, the ANOVA for 1975-1976 detected a significant treatment effect between the years of foliage, as did the ANOVA for the 1976 collection (Table 4.21). No significant effect for year of foliage origin was detected for the 1975 collection (Table 4.21, ANOVA B). The mean percent needle retention and 95% confidence intervals for plot E-1, both collections, are shown in Figure 4.13. The mean values for 1975 and 1974 years' foliage from plot E-1 are essentially the same, while that for 1973 year's foliage is nearly 12 percent lower. Whereas for the 1975 data, significant differences occurred usually only between the 1974 and 1972 internodes, the 1976 data generally show significant differences between 1973 and both 1974 and 1975 internodes.

For percent basal necrosis (Table 4.21) significant treatment effects were shown for 1975-1976 and 1975 between the years of foliage, but not for the 1976 collection. The mean values and 95% confidence intervals for basal

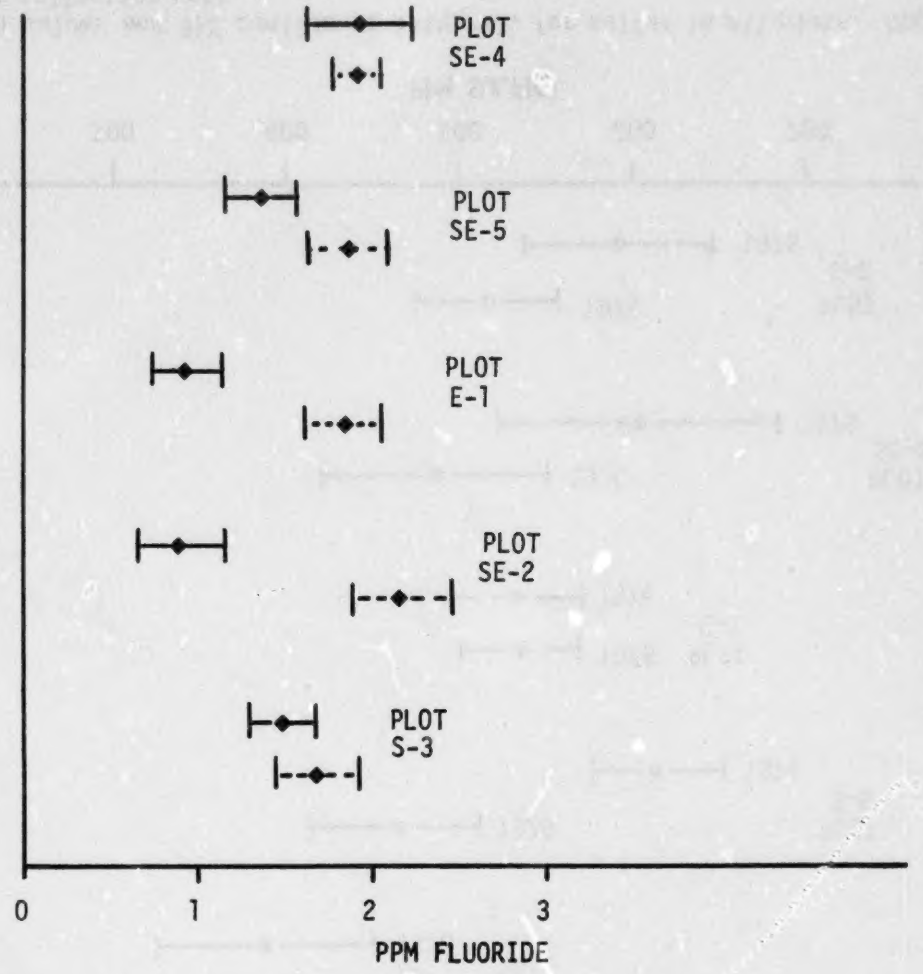

Figure 4.11. Mean values and 95% confidence intervals for fluoride in upper crowns, all years foliage combined, for all plots. 1975 (—◆—) and 1976 (--◆--) collections.

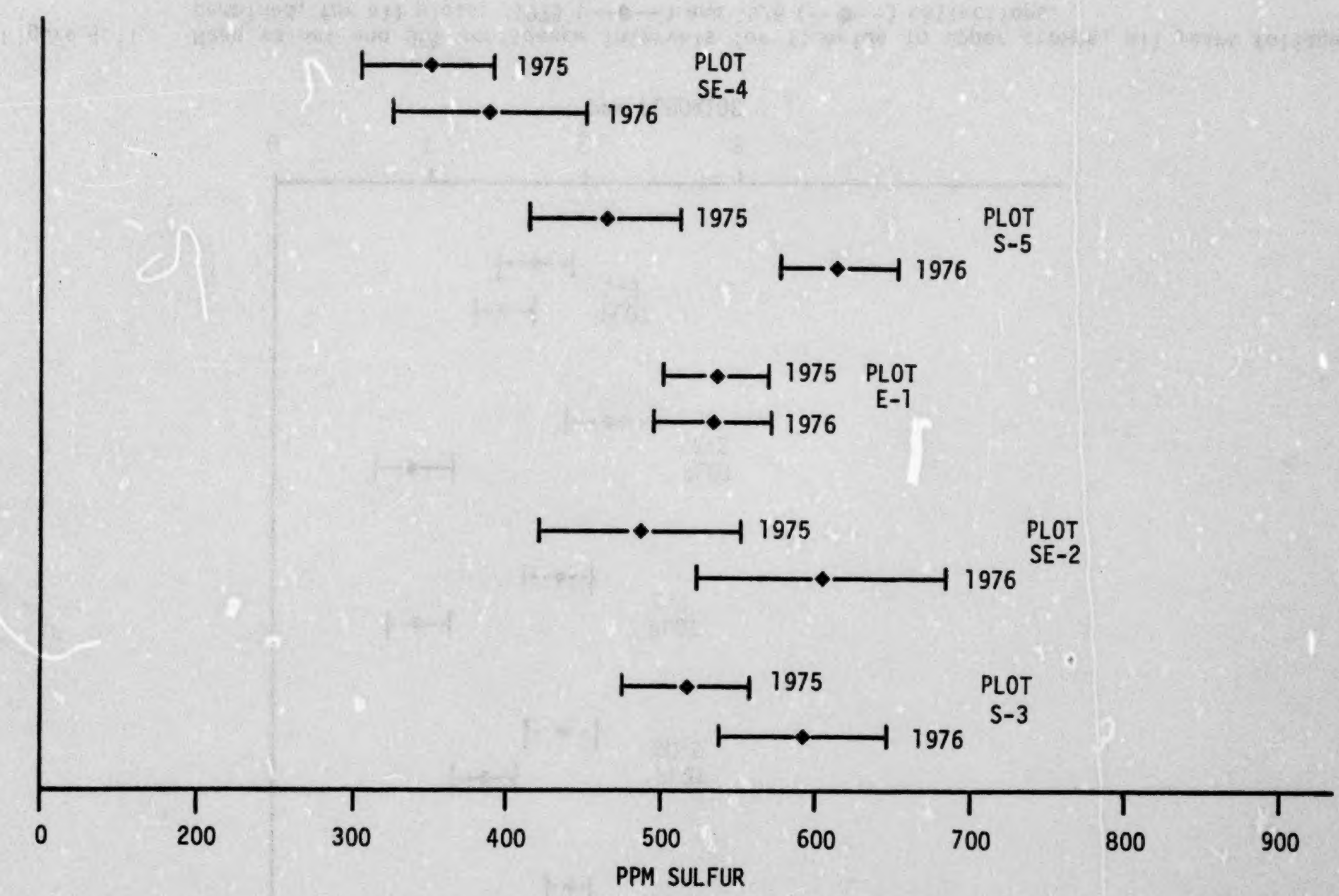

Figure 4.12. Mean values and 95% confidence intervals for sulfur in all plots. Upper crown, older trees, both collection sets.

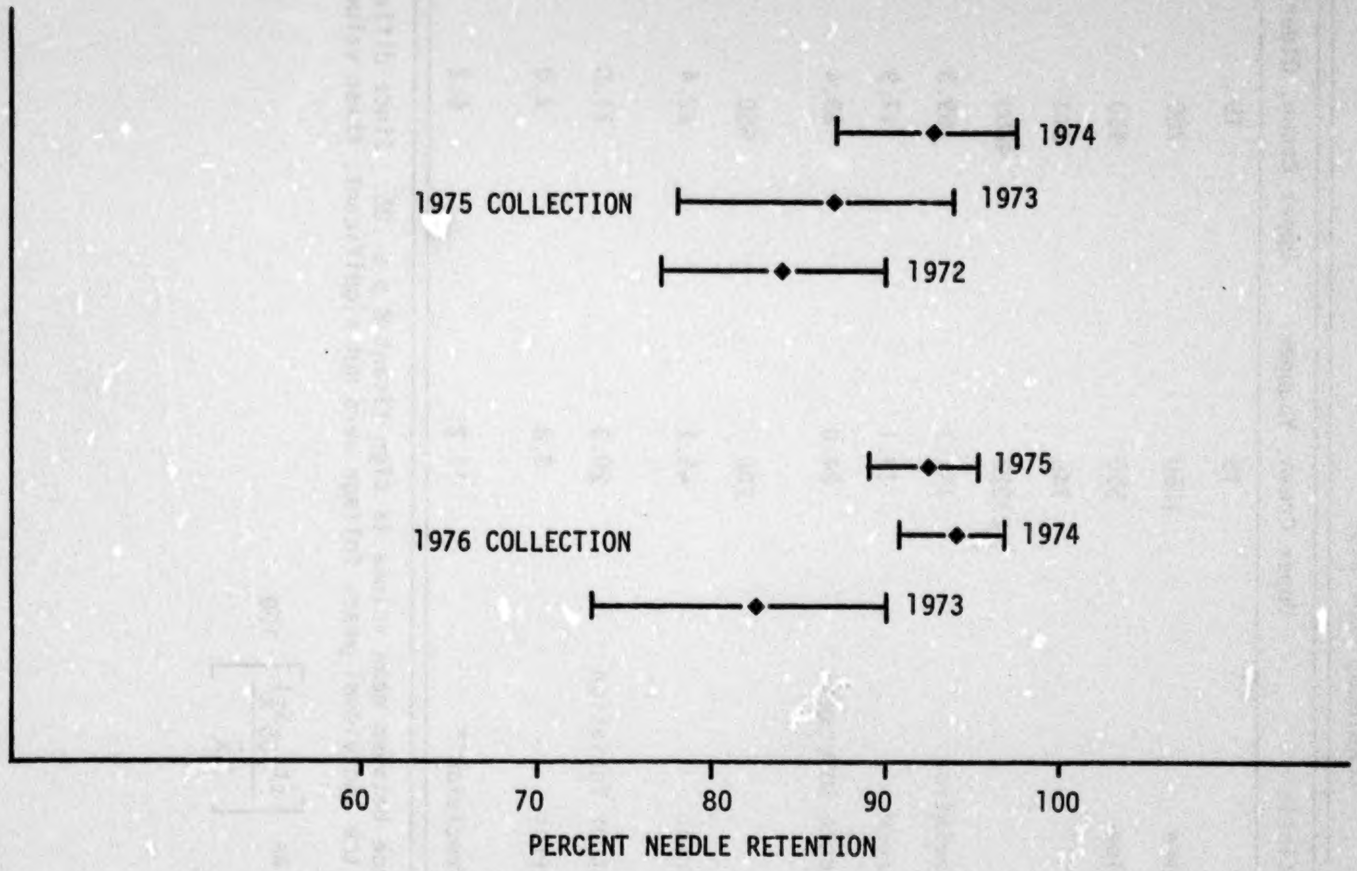

Figure 4.13. Mean values and 95% confidence intervals for percent needle retention, 1975 and 1976 collection, upper crown, for plot E-1.

TABLE 4.22. BASIC STATISTICS FOR SULFUR CONCENTRATIONS IN UPPER CROWN YOUNGER TREES AND UPPER CROWN OLDER TREES, ALL YEARS FOLIAGE COMBINED, PLOT S-5*

| Statistic | Upper Crown, Younger | Upper Crown, Older |
|---|---|---|
| N | 15 | 15 |
| Maximum Value | 1050 | 700 |
| Minimum Value | 550 | 450 |
| Mean | 746 | 613 |
| Variance | 23023 | 4809 |
| Standard Deviation | 151.7 | 69.3 |
| Standard Error | 39.1 | 17.9 |
| 95% Confidence Interval | 84.0 | 38.4 |
| Median | 700 | 650 |
| Standard Error | 49.1 | 22.4 |
| Coefficient of Variation | 20.3 | 11.3 |
| Standard Error | 3.8 | 2.0 |
| Sampling Precision** | 11.2 | 6.2 |

*Difference between mean values is significant @ $p \leq .05$. Since differences between the individual years foliage were not significant, these values were pooled.

**Defined as $\left[\frac{(t_{.05} S_{\bar{x}})}{\bar{x}}\right] 100$

TABLE 4.23. MEAN VALUES FOR TOTAL SULFUR FROM UPPER CROWN YOUNGER AND OLDER TREES, ALL PLOTS, 1976 COLLECTION.

Younger Trees

| Plot & Mean Sulfur Concentration \ Plot & Mean Sulfur Concentration | S-3 | SE-2 | E-1 | S-5 |
|---|---|---|---|---|
| SE-4 | 640<br>*<br>410 | 620<br>*<br>410 | 528<br>*<br>410 | 746<br>*<br>410 |
| S-5 | 640<br>*<br>746 | 620<br>*<br>746 | 528<br>*<br>746 | |
| E-1 | 640<br>*<br>528 | 620<br>*<br>528 | | |
| SE-2 | 640<br>620 | | | |

Older Trees

| Plot & Mean Sulfur Concentration \ Plot & Mean Sulfur Concentration | S-3 | SE-2 | E-1 | S-5 |
|---|---|---|---|---|
| SE-4 | 590<br>*<br>388 | 603<br>*<br>388 | 533<br>*<br>388 | 613<br>*<br>388 |
| S-5 | 590<br>613 | 603<br>613 | 533<br>*<br>613 | |
| E-1 | 590<br>533 | 603<br>533 | | |
| SE-2 | 590<br>603 | | | |

*Indicates mean values shown are significantly different @ $p \leqq .05$

necrosis for upper crown positions for the 1975 and 1976 collections are shown in Figure 4.14. Although the mean values for 1974 year's foliage increased slightly in 1976 over 1975, the values for 1973 foliage have not (Figure 4.14).

For percent mottled needles, the ANOVA for 1975 showed significant treatment effects between the tree ages, while the ANOVAs for 1975-1976 and 1976 indicated significant treatment effects between the years of foliage, but the ANOVA for 1975 did not.

The mean values and 95% confidence intervals for percent mottled needles for the 1976 collections from plot S-3 are shown in Figure 4.15, which illustrates the lack of effect between the tree ages (compare Figure 4.9).

For percent total needle necrosis, the ANOVA for 1975-1976 detected significant treatment effects between the two different years' collections and between the years of foliage. The ANOVA for 1975 detected significant effects only between the tree ages. The ANOVA for 1976 did not indicate differences between the mean values for tree ages but did detect differences between the years of foliage. For the individual years of foliage, the 95% confidence intervals enclosed the mean values for the tree ages at plot E-1 (Figure 4.16) for the 1975 collection as well as the 1976 collection. The mean values and 95% confidence intervals for older and younger trees combined are shown in Figure 4.16 for both collections from plot E-1. The 1976 data in Figure 4.16 illustrate the gradual increase in percent total necrosis fo the other plots in 1976.

For percent healthy needles, the ANOVA for 1975-1976 detected significant treatment effects between the collections. The 1975-1976 ANOVA did not indicate differences between the plots, nor did it for the 1975 collections. The 1976 ANOVA detected significant treatment effects between the plots. All ANOVAs indicated significant differences between the years of foliage (Table 4.21). The mean values and 95% confidence intervals for percent healthy needles for all plots from the 1976 collection are shown in Figure 4.17.

The results of the analysis of the data from the 1975 collections demonstrated that samples collected from the upper or lower crown positions of individual trees to evaluate the pre-operational baseline conditions for the 12 characteristics measured herein may be considered samples which are representative of the individual tree as a whole, with the exception of fascicular cross-sectional area. However, if such samples were collected from geographically separate plots, from different tree ages within a plot, or from different ages of foliage, they cannot a priori be considered to be samples from the same population.

Furthermore, the results of the data analysis for the 1976 collection and the comparison of both years' collections show that, with the exception of fluoride, percent total needle necrosis, and percent healthy needles, the two collections represent samples from the same population, even though differences were indicated for certain variables within the two collections. For example, treatment effects between the plots for sulfur concentrations were noted in 1976 but not in 1975, and treatment effects between the years of foliage were noted in 1976 but not in 1975. These effects, however, were not

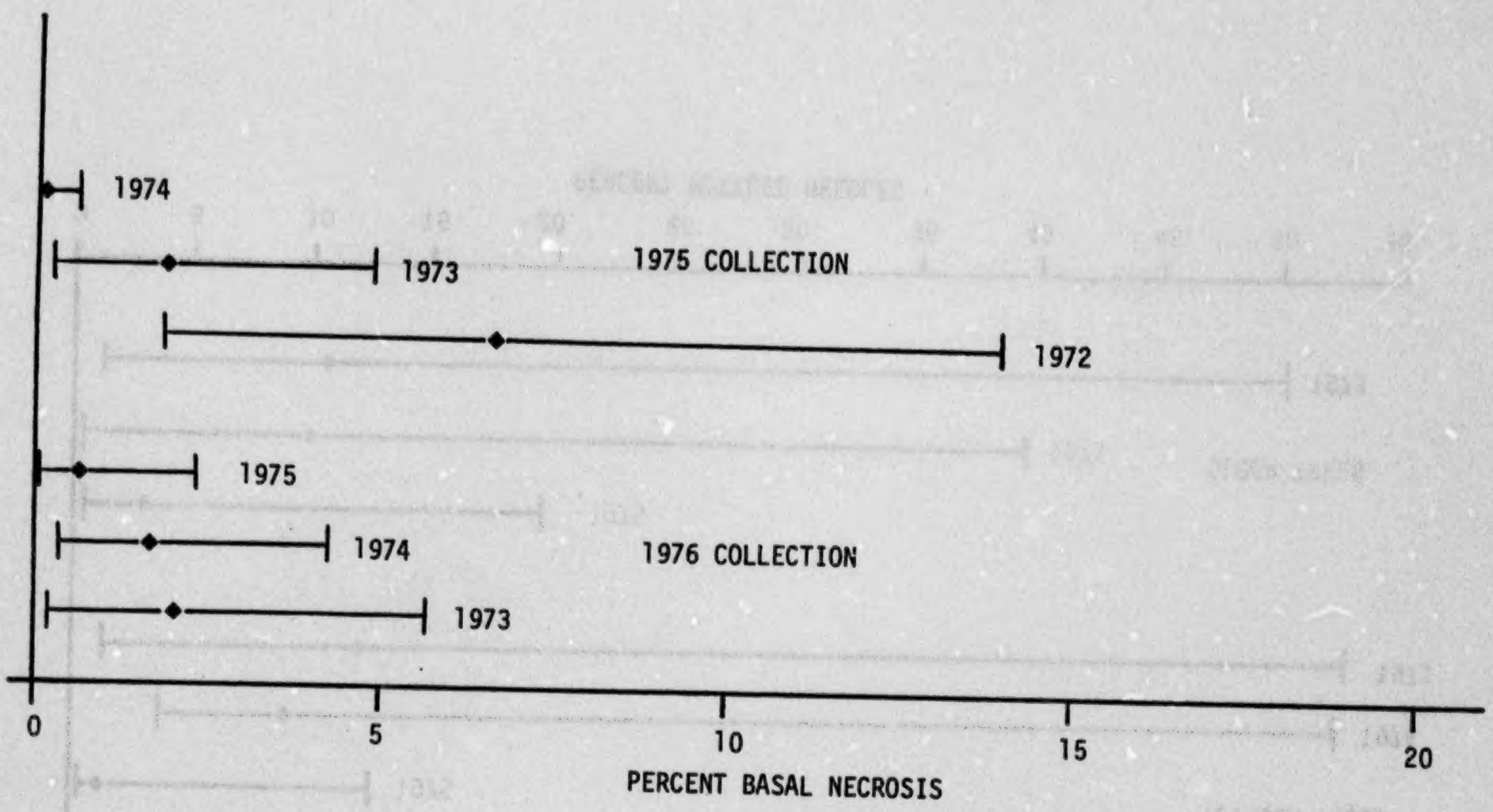

Figure 4.14. Mean values and 95% confidence intervals for percent basal necrosis, upper crown positions. 1975 and 1976 collections, plot E-1.

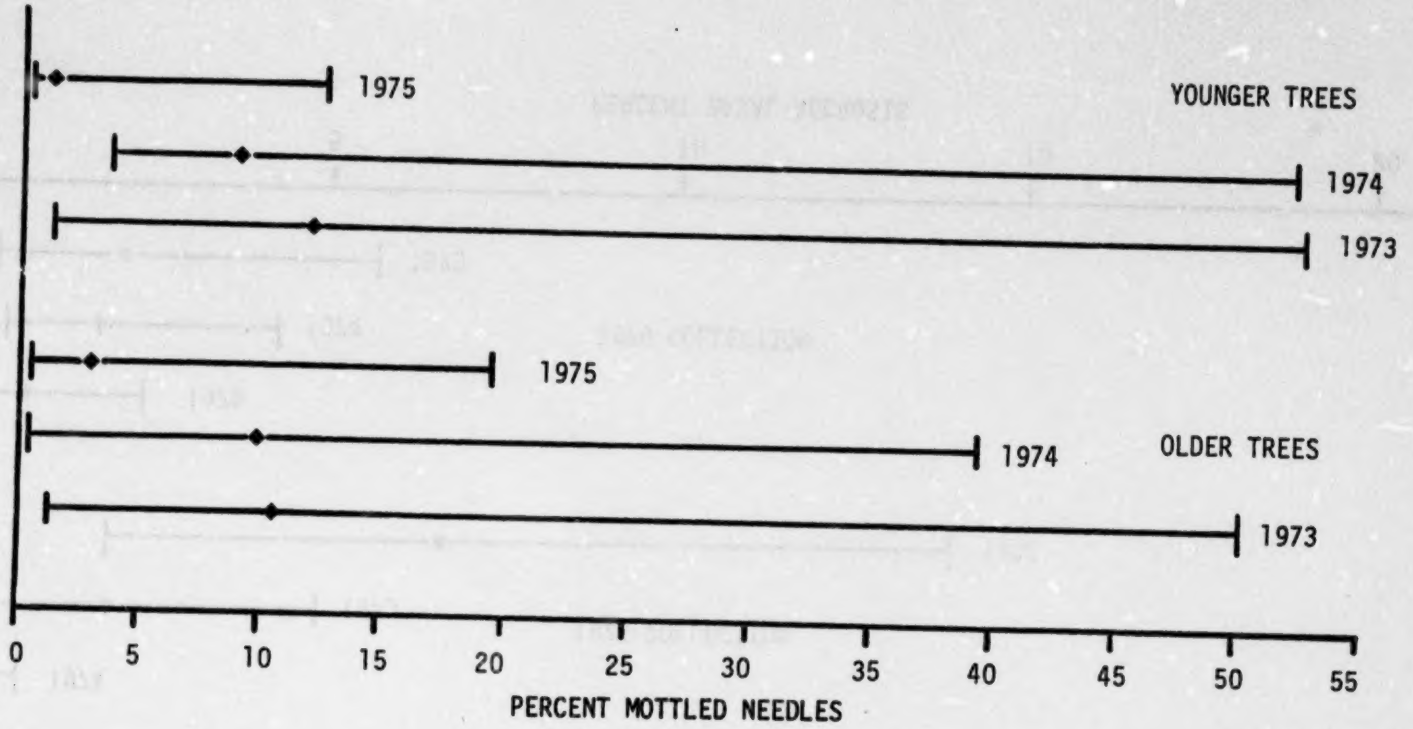

Figure 4.15. Mean values and 95% confidence intervals for percent mottled needles for upper crown, younger and older trees, plot S-3.

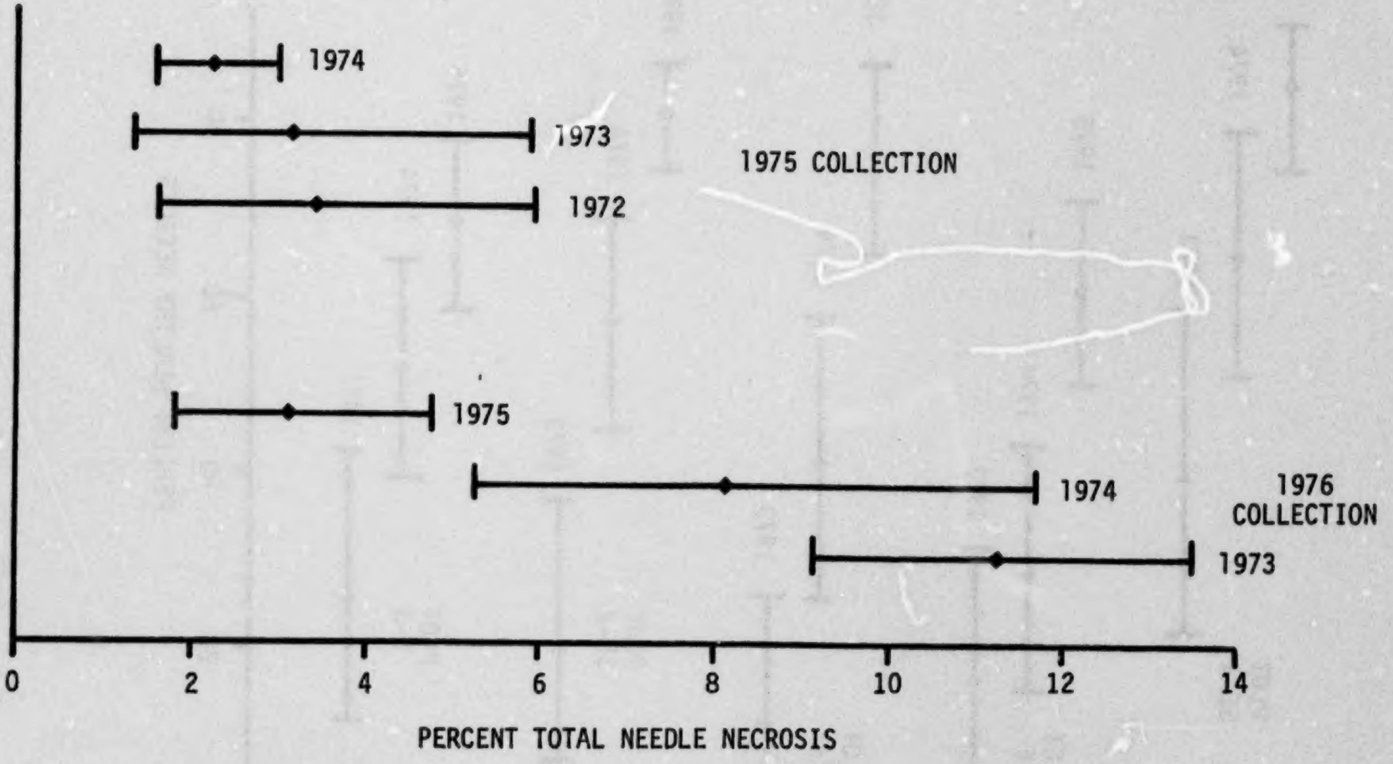

Figure 4.16. Mean values for percent total needle necrosis for upper crown, older and younger trees combined, 1975 and 1976 collections.

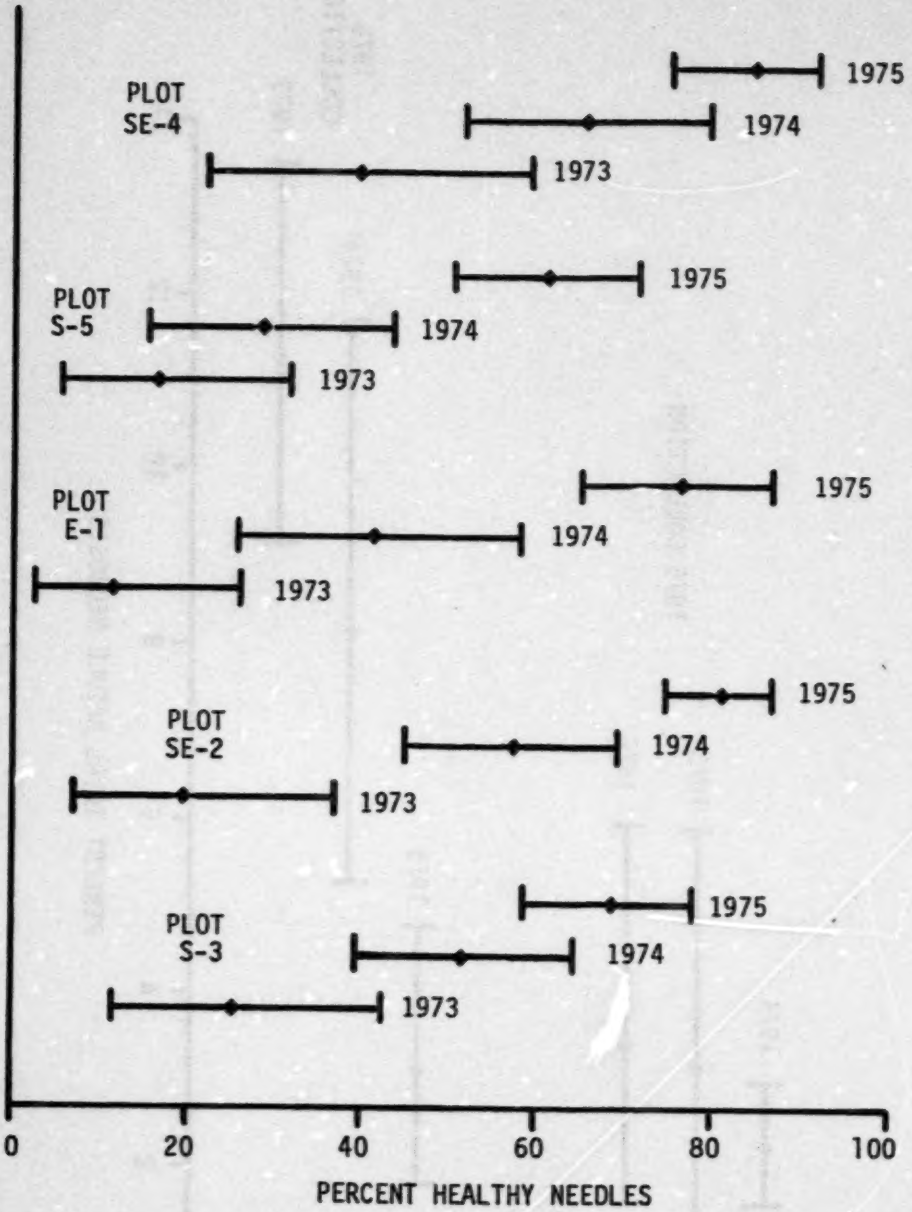

Figure 4.17. Mean values and 95% confidence intervals for percent healthy needles, 1976 collection.

sufficient to permit the conclusion that the two years' collections were samples from different populations.

## CHEMICAL ANALYSIS OF UNDERSTORY SPECIES

Table 4.24 depicts the results of fluoride and sulfur analyses and basic statistics for the foliage of nine understory species collected at the five permanent sites during both 1975 and 1976 collection periods. Also on this table are the results of fluoride and sulfur analyses of ten other understory species which were not abundant at all the ponderosa pine-skunkbush sites; these species were collected during either 1975 or 1976 at one or more of the five sites. The basic statistics for sulfur and fluoride content of these 19 total species is presented in Table 4.25. During 1977, eight separate samples of each understory species from each site will be collected for chemical analysis.

The mean fluoride concentration found for all understory species was below 5 ppm. Little bluestem and bluebunch wheatgrass, the two most common grass species at the ponderosa sites, usually had the lowest fluoride levels (i.e., < 2 ppm) of any understory species collected. Sulfur levels found in foliage of silver sage, broom snakeweed, and arrowleaf balsamroot usually were higher than other common understory species and two to three times higher than the two commonly found grass species.

In a study of fluoride- and sulfur-polluted areas in which ponderosa pine foliage and understory species of skunkbush, broom snakeweed, and bluebunch wheatgrass were utilized, Gordon et al. (1977) demonstrated that the foliage of the dominant species (ponderosa pine) accumulates excessive levels of both sulfur and fluoride at a faster rate than the understory species during an exposure time of 15 months. The researchers do not anticipate any significant increase of fluoride or sulfur accumulation in understory species at the five ponderosa pine-skunkbush sites until the ponderosa pine foliage reaches two to three times the baseline for fluoride and 1.5 times the baseline for sulfur.

## DISCUSSION AND SUMMARY

### SULFUR

Several past studies of air pollution impacts on conifer species (i.e., Linzon, 1973a; Dreisinger, 1965; Ellertsen et al., 1972; Berry et al., 1964; Miller et al., 1963; Carlson, 1972) have encompassed one to four of the growth/health/disease characteristics (i.e., tip burn, needle retention, needle length, and percent necrosis) which have been used in the Colstrip ponderosa pine sites during the last two years to quantify the variability of these characteristics prior to the incidence of air pollution damage. Also, there have been several air pollution studies of conifer trees to determine sulfur and/or fluoride concentrations in damaged and undamaged foliage (i.e., Katz et al., 1952; Carlson, 1974; Compton et al., 1961; Ellertsen et al., 1972; Knabe, 1968; Linzon, 1972, 1973b).

Linzon's sulfur studies in the Sudbury area of Ontario revealed that 15-month-old jack pine foliage (Pinus banksiana), growing 27 km northeast and 160

TABLE 4.24. FLUORIDE AND SULFUR CONCENTRATIONS IN UNDERSTORY SPECIES FROM THE 1975 AND 1976 COLLECTIONS.

| Species | SE-4 | | S-5 | | E-1 | | SE-2 | | S-3 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | ppm F$^-$ 1975 1976 | ppm S 1975 1976 | ppm F$^-$ 1975 1976 | ppm S 1975 1976 | ppm F$^-$ 1975 1976 | ppm S 1975 1976 | ppm F$^-$ 1975 1976 | ppm S 1975 1976 | ppm F$^-$ 1975 1976 | ppm S 1975 1976 |
| Silver Sage | 2.3<br>2.3 | 1300<br>1950 | 3.0<br>3.0 | 1300<br>1550 | 1.3<br>1.8 | 1300<br>1400 | 2.0<br>2.7 | 1400<br>1250 | ---<br>2.5 | ----<br>850 |
| Broom Snakeweed | ---<br>1.7 | ----<br>950 | 1.5<br>--- | 1675<br>---- | ---<br>--- | ----<br>---- | 2.2<br>4.9 | 1800<br>2500 | ---<br>2.3 | ----<br>1200 |
| Chokecherry | 1.8<br>2.4 | 650<br>800 | ---<br>--- | ----<br>---- | ---<br>--- | ----<br>---- | 2.5<br>--- | 600<br>---- | 0.7<br>4.7 | 300<br>500 |
| Skunkbush | 6.4<br>0.9 | 950<br>900 | 1.9<br>1.7 | 950<br>950 | 1.8<br>--- | 1800<br>700 | 4.1<br>4.9 | 800<br>1000 | 3.6<br>2.0 | 600<br>600 |
| Idaho Fescue | 0.8<br>1.4 | 700<br>950 | ---<br>2.0 | ----<br>800 | ---<br>--- | ----<br>---- | ---<br>--- | ----<br>---- | ---<br>2.2 | ----<br>550 |
| Bluebunch Wheatgrass | 1.1<br>1.7 | 800<br>950 | 2.8<br>0.9 | 1100<br>750 | 1.1<br>--- | 500<br>---- | 2.1<br>2.1 | 600<br>600 | ---<br>2.5 | ----<br>700 |
| Prairie Sage | 2.2<br>3.1 | 900<br>1000 | 5.7<br>4.0 | 1150<br>1000 | 0.9<br>--- | 1000<br>---- | 4.2<br>--- | 1100<br>---- | 4.0<br>--- | 650<br>---- |
| Arrowleaf Balsamroot | ---<br>4.0 | ----<br>2400 | 4.9<br>4.1 | 1150<br>750 | ---<br>--- | ----<br>---- | ---<br>--- | ----<br>---- | ---<br>--- | ----<br>---- |
| Little Bluestem | ---<br>--- | ----<br>---- | ---<br>--- | ----<br>---- | 2.5<br>3.3 | 300<br>250 | 2.3<br>1.2 | 400<br>300 | 1.2<br>1.0 | 300<br>400 |
| Lupine | ---<br>2.1 | ----<br>1400 | 2.1<br>2.0 | 1100<br>950 | ---<br>5.5 | ----<br>1150 | ---<br>2.1 | ----<br>950 | 4.9<br>3.4 | 750<br>800 |

(continued)

TABLE 4.24. (continued)

| Species | SE-4 | | S-5 | | E-1 | | SE-2 | | S-3 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | ppm $F^-$ 1975 1976 | ppm S 1975 1976 | ppm $F^-$ 1975 1976 | ppm S 1975 1976 | ppm $F^-$ 1975 1976 | ppm S 1975 1976 | ppm $F^-$ 1975 1976 | ppm S 1975 1976 | ppm $F^-$ 1975 1976 | ppm S 1975 1976 |
| Scurf Pea | --- | ---- | --- | ---- | 2.8 | 950 | 1.9 | 900 | --- | ---- |
| | --- | ---- | --- | ---- | 4.7 | 1350 | --- | ---- | 2.9 | 900 |
| Yucca | --- | ---- | 2.8 | 800 | --- | ---- | --- | ---- | --- | ---- |
| | --- | ---- | 1.9 | 700 | 5.6 | 750 | --- | ---- | 1.6 | 650 |
| Red Three-Awn | --- | ---- | --- | ---- | 2.9 | 500 | --- | ---- | --- | ---- |
| | --- | ---- | --- | ---- | 1.7 | 650 | --- | ---- | --- | ---- |
| Needle and Thread | --- | ---- | --- | ---- | 2.7 | 700 | 2.2 | 1100 | --- | ---- |
| | --- | ---- | --- | ---- | --- | ---- | 4.4 | 1200 | --- | ---- |
| Vetch | --- | ---- | --- | ---- | 2.4 | 1200 | --- | ---- | --- | ---- |
| | --- | ---- | --- | ---- | --- | ---- | --- | ---- | --- | ---- |
| Fringed Sage | --- | ---- | --- | ---- | --- | ---- | --- | ---- | --- | ---- |
| | 2.6 | 1400 | 2.4 | 1250 | 5.3 | 1250 | 1.8 | 1100 | 1.9 | 1200 |
| Green Rabbit Brush | --- | --- | --- | ---- | --- | ---- | --- | ---- | --- | ---- |
| | --- | ---- | 2.0 | 1550 | --- | ---- | --- | ---- | --- | ---- |
| Rocky Mountain Juniper | --- | ---- | --- | ---- | --- | ---- | --- | ---- | --- | ---- |
| | --- | ---- | --- | ---- | --- | 600 | 1.9 | 800 | --- | ---- |
| Big Sagebrush | --- | ---- | --- | ---- | --- | ---- | --- | ---- | --- | ---- |
| | --- | ---- | --- | ---- | --- | ---- | 2.4 | 1300 | --- | ---- |

TABLE 4.25. UNDERSTORY SPECIES, PONDEROSA PINE - SKUNKBUSH SITES.

| Species and Year of Collection | | Mean | | Standard Deviation | | Standard Error | |
|---|---|---|---|---|---|---|---|
| | | $\bar{F}$ | S | $\bar{F}$ | S | $\bar{F}$ | S |
| Silver Sage | 1975 | 2.15 | 1230 | .70 | 216 | .35 | 96.7 |
| | 1976 | 1.85 | 1400 | .49 | 403 | .35 | 180.2 |
| Broom | 1975 | 1.85 | 1737 | .49 | 88.3 | .35 | 62.5 |
| Snakeweed | 1976 | 2.97 | 1550 | 1.7 | 832.1 | .98 | 480.4 |
| Chokecherry | 1975 | 1.67 | 516 | .91 | 189 | .52 | 109 |
| | 1976 | 3.55 | 650 | 1.63 | 212 | 1.15 | 150 |
| Skunkbush | 1975 | 3.56 | 1020 | 1.88 | 459 | .84 | 205.3 |
| | 1976 | 2.38 | 830 | 1.75 | 171.7 | .87 | 76.8 |
| Bluebunch | 1975 | 1.78 | 750 | .83 | 264 | .42 | 132.2 |
| Wheatgrass | 1976 | 1.8 | 750 | .68 | 147.2 | .34 | 73.6 |
| Prairie Sage | 1975 | 3.4 | 960 | 1.87 | 198.1 | .84 | 88.6 |
| | 1976 | 3.55 | 1000 | .64 | 0 | .45 | 0 |
| Little | 1975 | 2.0 | 333 | .7 | 57.7 | .40 | 33.3 |
| Bluestem | 1976 | 1.83 | 316 | 1.27 | 76.3 | .73 | 44.0 |
| Lupine | 1975 | 3.5 | 925 | 1.97 | 247 | 1.4 | 175 |
| | 1976 | 3.0 | 1050 | 1.5 | 231.1 | .67 | 103.6 |
| Scurf Pea | 1975 | 2.35 | 925 | .63 | 35.3 | .45 | 25 |
| | 1976 | 3.8 | 1125 | 1.27 | 318 | .9 | 225 |
| Idaho | 1975 | .8 | 700 | ---- | --- | ---- | --- |
| Fescue | 1976 | 1.87 | 766 | .42 | 202 | .24 | 116.6 |
| Arrowleaf | 1975 | 4.9 | 1150 | ---- | --- | ---- | --- |
| Balsamroot | 1976 | 4.05 | 1575 | .07 | 1166 | .05 | 825 |
| Yucca | 1975 | 2.8 | 800 | ---- | --- | ---- | --- |
| | 1976 | 3.03 | 700 | 2.23 | 50 | 1.29 | 28.8 |
| Red Three-Awn | 1975 | 2.9 | 500 | ---- | --- | ---- | --- |
| | 1976 | 1.7 | 650 | ---- | --- | ---- | --- |
| Needle-and- | 1975 | 2.45 | 900 | .35 | 282 | .25 | 200 |
| Thread | 1976 | 4.4 | 1200 | ---- | --- | ---- | --- |
| Vetch | 1975 | 2.4 | 1200 | ---- | --- | ---- | --- |
| Fringed Sage | 1976 | 2.8 | 780 | 1.44 | 713.6 | .64 | 318.1 |
| Common Rabbitbrush | 1976 | 2.0 | 1550 | ---- | --- | ---- | --- |
| Rocky Mountain Juniper | 1975 | 1.9 | 700 | ---- | 141.4 | ---- | 100 |
| Big Sage | 1976 | 2.4 | 1300 | ---- | --- | ---- | --- |

km west of the non-ferrous smelters, contained sulfur averaging 2,200 to 1,000 ppm, respectively. Jack pine foliage of the same age collected at two intermediate-distance sampling sites, 45 and 67 km northeast, had average sulfur concentrations of 1,700 and 1,300 ppm, respectively, while three-month-old foliage from these same two intermediate sites had average sulfur concentrations of 1,500 to 1,400 ppm respectively.

Katz et al. (1952) worked with both field-grown and potted ponderosa pines and fumigated with $SO_2$ concentrations of 22 to 500 pphm for durations of 35 to 1,656 hours (1.4 to 69 possible day periods), respectively. These studies revealed that the most elevated sulfur levels (5,600 ppm) were found in one-year-old foliage exposed to the lowest concentration (22 pphm) for the longest period of time (1,656 hours).

Katz's fumigation studies also revealed that one-, two-, and three-year-old ponderosa pine foliage fumigated for 96 hours with $SO_2$ concentrations of 500 pphm contained 120, 40, and 300 ppm of sulfur, respectively. In these studies, there appeared to be a relationship between excessive sulfur accumulation and the incidence of the $SO_2$-caused needle necrosis that Katz was attempting to determine. For instance, ponderosa pine trees fumigated with 500 pphm of sulfur for 96 hours developed no foliage necrosis but had 530 ppm of sulfur in the one-year-old needles and 520 ppm in the two-year-old needles after the fumigation. In another $SO_2$ fumigation study utilizing 500 pphm for 40 hours, Katz found that 100 percent necrosis occurred, and in the one-, two-, and three-year-old foliage, the sulfur levels were found to be elevated above 750 ppm.

In all of Katz's, as well as Linzon's (1958 to 1973) reported $SO_2$ field and fumigation studies, no details were presented on the variation of sulfur concentrations in healthy foliage of pines within and between the different years' growth. Thus, it is difficult to compare the variability in sulfur concentrations of ponderosa pine from the Colstrip study area with either of their studies.

Another field study in the area of the Trail, B.C., smelter (besides that of Katz) was carried out by U.S. Forest Service personnel (Sheffer and Hedgcock 1955) between 1928 and 1936. One of the forest species collected and studied by these two investigators was ponderosa pine. Sulfur analysis of damaged and undamaged ponderosa pine foliage revealed that foliage with concentrations of 2,700 to 2,300 ppm collected at sites 18 and 37 km, respectively, south of the emission source were damaged but that foliage with 2,000 to 1,600 ppm collected 42 to 70 km, respectively, south of the source were undamaged. Baseline sulfur content of healthy ponderosa pine foliage collected by Scheffer and Hedgcock 79 to 97 km south of the source was found to range from 800 to 1,000 ppm. Over 25,000 ponderosa pine trees were utilized in this eight-year field study, and the various types of damage include decreased cone production and seedling reproduction, loss of annual increment, premature needle casting, and increased sulfur content of the foliage. This study was one of the first and only field studies in which the investigators set up their studies and carried them out to compare and understand the variabilities of pathologies at control sites versus the impact sites.

Some field investigations during the last 15 years, such as Carlson's (1974) study on damage to ponderosa pine from a small sulfur dioxide source in the Missoula Valley of Montana, demonstrated that elevated sulfur content of pine (Pinus ponderosa) is related to the severity of the foliage damage; other studies, such as those by Ellertsen (1972) and Berry and Hepting (1964) on sulfur and fluoride levels in damaged white pine (Pinus strobus) foliage in Tennessee, did not demonstrate this.

To date, after an intensive search through the sulfur dioxide pollution literature involving both field and controlled fumigation chamber investigations, the investigators have not found any studies dealing with $SO_2$ damage to plants that quantify the variability of baseline sulfur content within foliage of any conifer species between different years' foliage or between foliage from different control sites. However, numerous field investigators and some control fumigation investigators have utilized the sulfur content of $SO_2$-damaged foliage as one of the major indices of their pollution studies. If the variability of the sulfur content of foliage from controlled areas is not known, then sulfur levels in damaged foliage is much less useful for understanding if and when airborne sulfur emissions are affecting ecosystems.

Every field investigator mentioned above used at least one foliage pathology or characteristic to demonstrate air pollution impact on the study sites. Also, each was studying an area which was being severely impacted by the emissions from one or more large stationary sources. Investigations in areas where acute fumigation damage is occurring may not require an understanding of the baseline quantities of needle pathologies or the variability of those pathologies between different years' foliage, different-sized trees, different crown positions, and different sites. However, these baseline quantities are absolutely essential for understanding if and where chronic air pollution damage is occurring within ecosystems.

The baseline studies on the ponderosa pine sites clearly demonstrated that abiotic and biotic causal agents normally occurring in a pristine area cause a measurable amount of damage to pine foliage; this damage has to be quantified prior to understanding if and where chronic air pollution damage is occurring.

## FLUORIDE

Fluoride studies from both field and fumigation chamber investigations have revealed that this element is the most phytotoxic of the normal gaseous emissions from large stationary sources of air pollution (Lione et al., 1962). More controlled $SO_2$ fumigation studies have been completed and published than fluoride studies, but there are more published field studies dealing with fluoride pollution than with any other phytotoxic gas because: (1) Gaseous and particulate fluorides are the major emissions of large stationary sources such as aluminum and steel smelters, and phosphate fertilizer, animal supplement, and $P_2O_5$ plants; (2) in most cases, baseline fluoride levels in vegetation are below 10 ppm, and fluorine has not proven to be an essential ion for plant metabolism or structure, and (3) fluoride pollution problems, in many past instances, have involved domestic animals or agricultural crops, provoking intensive scientific investigation.

Fluoride is not a major pollutant within the emissions of coal-fired power plants or non-ferrous smelters, and few studies around either pollution source have included this element or compound. However, because of the phytotoxicity of even low concentrations (.5 ppb) of fluoride in the ambient air to plant pollen (Facteau *et al.*, 1973, 1977), its low translocation rate from the leaf receptor to other parts of the plant (thus remaining in the leaf), and its rapid build-up in the food chain, the investigators have spent considerable time determining the baseline levels of this element in the ponderosa pine-skunkbush ecosystems of the Colstrip area.

The fluoride studies in upper crown foliage at the ponderosa sites were set up to best utilize the evidence gained by Knabe (1968) in forest canopy fluoride studies which demonstrated that the most elevated fluorides in coniferous foliage were in the highest and most exposed upper crown foliage. Field studies in the vicinity of aluminum plants by various investigators (Shaw *et al.*, 1951; Adams *et al.*, 1952; Compton *et al.*, 1961; Facteau *et al.*, 1976; Carlson, 1972; Gordon, 1974) in the United States have demonstated that ponderosa pine is very susceptible to fluoride pollution. Current and one-year-old foliage of ponderosa pine with fluoride levels less than 20 ppm have been reported to manifest needle tip necrosis by Compton *et al.* (1968), Carlson (1972), Hindawi (1973), and Gordon *et al.* (1976).

Partitioned ponderosa pine needles damaged by fluoride pollution have been reported by Gordon *et al.* (1976) to contain up to three times more fluoride in the 3 cm tip portion than whole needles and over six times more fluoride in the needle tips than middle and basal portions. Both Jacobson *et al.* (1966) and Compton *et al.* (1960) have demonstrated with fluoride-fumigated gladiolus plants that after fluoride enters the leaves, it is translocated to the leaf tips where tissue necrosis then occurs. Jacobson's (1966) fluoride fumigation studies with other species of plants (cotton and tomato) to determine fluoride translocation and partitioning in plant tissues led him to suggest that the ability of a plant to readily translocate and partition fluoride may influence its susceptibility or resistance to fluoride damage.

The 1976 data from this study demonstrated an increase in fluoride concentrations in ponderosa pine foliage from Kluver (E-1), McRae (SE-2), and Fort Howes, Custer National Forest (S-5) sites over that occurring in foliage of the same age during 1975. This was not accompanied by any significant increase in sulfur levels in the needles at these three sites between the 1975 and 1976 study periods. Because sulfur taken in from the ambient air is translocated out of the pine needles, no significant increase is expected at any of these sites until after the emissions of the Colstrip coal-fired power plant units increase from the estimated average 1976 level of .8 tons per hour to the higher potential levels of +2 tons per hour.

## NEEDLE PATHOLOGIES

While there was a significant increase in both the amount of total needle necrosis and loss of healthy needles at all the ponderosa pine-skunkbush sites during 1976 over that of 1975, at this time the increase is not attributed to phytotoxic emissions of Colstrip units. Houston (1974), fumigating white pine with a combination of $SO_2$ (.025 ppm) and $O_3$ (.05 ppm) for six hours, reported that all sensitive clones manifested tip necrosis on 75 to 100 percent of current year needles. However, these are hypersensitive clones of white pine and are much more sensitive to both of these gases than ponderosa pine (Miller et al., 1975). Constantindou et al. (1976) reported a significant decrease in the chlorophyll content of red pine (Pinus resinosa) cotyledons when fumigating with 0.5 ppm $SO_2$ for both 15-and 30-minute periods. Kress and Skelly (1977), when fumigating loblolly pine (Pinus taeda) with $SO_2$ (0.14 ppm) + $O_3$ (.05 ppm) + $NO_2$ (0.10 ppm) for six hours per day for 28 consecutive days reported a 26 percent reduction in growth. The studies of both Constantindou et al. and Kress and Skelly support the current belief that it is unlikely that ponderosa pine foliage will be significantly impacted until the emissions are increased from the Colstrip units or until the plume directly impacts the Kluver (E-1) or McRae (SE-2) sites.

From the annual $SO_2$ concentrations at the DHES Anaconda monitoring sites (.004 to .02 ppm) as well as the forest-damage studies in Erzgebirge, Germany, reported by Materna (1973) where the annual $SO_2$ concentrations ranged from .006 to .009 ppm (6 to 9 ppb), it is believed that ponderosa pine needle pathologies will be the first significant manifestations of an increase in phytotoxic gases in the Colstrip area.

A table has been prepared in Appendix A which presents the results of $SO_2$ control fumigation and field studies during the last few years on conifer species. This information demonstrates that low $SO_2$ levels alone or in combination with other phytotoxic gases ($O_3$ and/or $NO_2$) for short durations can cause damage to conifer species.

## CONCLUSIONS

The results of these studies show that the assessment of the impacts of chronic air pollution damage requires the measurement of baseline characteristics and the monitoring of changes in these characteristics over time. This is clear from the changes found in fluoride, percent total needle necrosis, and percent healthy needles between 1975 and 1976. In addition to demonstrable changes between collections, the detection of subtle changes within succeeding collections is necessary to make predictions of future air pollution damage.

In general, the field studies mentioned in the preceding discussion have attempted to measure conditions existing in areas subjected to air pollution, but only after impact has occurred, and usually without baseline data other than that from control areas isolated geographically from the impacted study sites. Although such studies have generated useful data, their value as quantitative predictors of air pollution damage to ecosystems are limited by their study designs.

In a pragmatic sense, hard data which predict the effects of air pollution on ecosystems are required for input into those decision-making processes regarding the siting of stationary sources of air pollution. This study was designed to be predictive of air pollution impacts to forest ecosystems in the Northern Great Plains for input into such decision-making processes. The results thus far indicate that this design is sufficiently sensitive to predict and to detect air pollution impacts to forest ecosystems in this area.

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## APPENDIX A

### LESS THAN ONE-HOUR EXPOSURE OF $SO_2$ ALONE AND IN COMBINATION WITH OTHER POLLUTANTS

| | TIME | CONCENTRATION | RESPONSE OF VEGETATION | PLANT SPECIES | AUTHOR |
|---|---|---|---|---|---|
| 1. | 15 min. | .5 ppm $SO_2$ | Significant decrease in chlorophyll content of cotyledons. Significant decrease in chlorophyll content of cotyledons at 1, 3, and 4 ppm. Highly significant reductions in chlorophyll in primary needles at .5, 1, 3, and 4 ppm.<br>Highly significant decrease in increment of total dry weight (growth)of cotyledons at .5, 1, 3, and 4 ppm. Highly significant decrease in increment of total dry weight (growth) of primary needles at .5, 1, and 3 ppm; significant decrease reduction at 4 ppm.<br>No necrosis of tips of cotyledons and primary needles at 1 day after treatment of .5, 1, 3, or 4 ppm. | Red pine seedlings<br>*Pinus resinosa* | Constantindou *et al*., 1976 |
| 2. | 30 min. | .5 ppm $SO_2$ | Significant decrease in chlorophyll content of cotyledons. Highly significant decrease in chlorophyll content of cotyledons at 1, 3, and 4 ppm.<br>Highly significant decrease in chlorophyll content of primary needles at .5, 1, 3, and 4 ppm.<br>Highly significant decrease in increment of total dry weight (growth) of cotyledons at .5, 1, 3, and 4 ppm. Highly significant decrease in increment of total dry weight (growth) of primary needles at .5 and 1 ppm; significant at 3 and 4 ppm. | Red pine seedlings<br>*Pinus resinosa* | Constantindou *et al*., 1976 |

APPENDIX (continued)

| | TIME | CONCENTRATION | RESPONSE OF VEGETATION | PLANT SPECIES | AUTHOR |
|---|---|---|---|---|---|
| | | | 37% of seedlings had necrosis of tips of cotyledons and primary needles 1 day after exposure to 4 ppm. | | |
| 3. | 1 hour | .5 ppm $SO_2$ | Significant decrease in chlorophyll content of cotyledons. Highly significant decrease in chlorophyll content of cotyledons at 1, 3, and 4 ppm. | Red pine seedlings<br>*Pinus resinosa* | Constantindou *et al.*, 1976 |
| | | | Highly significant decreases in chlorophyll of primary needles at .5 and 1 ppm; significant decreases at 3 and 4 ppm. | | |
| | | | Highly significant decrease in increment of total dry weight (growth) of cotyledons at 3 and 4 ppm. Exposures .5 and 1 ppm were significant. Highly significant decreases in increment of total dry weight (growth) of primary needles at .5 and 1 ppm; significant at 3 and 4 ppm. | | |
| | | | 15% of seedlings had necrosis of tips of cotyledons and primary needles 1 day after exposure to 3 ppm; 25% had necrosis after exposure to 4 ppm. "Continuous fumigation with $SO_2$ at much lower dosages than used may have inhibitory effects on seedling development and on regeneration of plant communities." | | |

APPENDIX (continued)

| | TIME | CONCENTRATION | RESPONSE OF VEGETATION | PLANT SPECIES | AUTHOR |
|---|---|---|---|---|---|
| 4. | 15 min. to 2 hours. | .5 ppm $SO_2$ | Highly significant decrease in chlorophyll content of cotyledons for .5, 1, 3, and 4 ppm.<br>Highly significant decreases in chlorophyll content in primary needles.<br>Highly significant decrease in increment of total dry weight at 3 and 4 ppm. Exposures to .5 and 1 ppm were significant.<br>Highly significant decrease in increment of total dry weight (growth) of primary needles at .5 and 1 ppm; significant at 3 and 4 ppm.<br>15% of seedlings had necrosis of tips of cotyledons and primary needles 1-day after exposures to 3 ppm; 35% after exposure to 4 ppm. | Red pine seedlings<br>*Pinus resinosa* | Constantindou *et al.*, 1976 |
| 5. | 2 hours | 0.25 ppm $SO_2$ | 6.5% of exposed parts injured. Needle lesions. | Eastern white pine<br>*Pinus strobus* | Berry, 1971 |
| 6. | 2 hours | 0.25 ppm $SO_2$ | 4.5% of exposed parts injured. Needle lesions. | Jack pine<br>*Pinus banskiana* | Berry, 1971 |
| 7. | 2 hours | 0.05 ppm $SO_2$ | Caused tip necrosis on new needles. | East. White pine<br>*Pinus strobus* | Costonis, 1973 |

APPENDIX (continued)

|  | TIME | CONCENTRATION | RESPONSE OF VEGETATION | PLANT SPECIES | AUTHOR |
|---|---|---|---|---|---|
|  |  | Alone |  |  |  |
|  | 2 hours | 0.04 ppm Followed 3 min. later by 0.05 ppm $SO_2$ for 2 hrs. and then fumigated 24 hrs. later with a mixture at same concentration. | [Needle necrosis (author stated necrosis was most severe at this exposure).] |  |  |
| 8. | 4 hours | 0.05 ppm $SO_2$ + 0.05 ppm ozone | Severe needle necrosis (2-3 cm leaf tip injured) on new needles, expressed most vividly 72 hours after exposure. | East. White pine *Pinus strobus* | Costonis, 1973 |
|  |  |  | $SO_2$ IN COMBINATION WITH OTHER POLLUTANTS |  |  |
| 9. | 6 hours per day for 28 days | 0.14 ppm $SO_2$ + 0.05 ppm ozone + 0.10 ppm $NO_2$ | Significant growth reduction (measured as height) compared to either ozone and sulfur dioxide combined, or ozone alone. Needles were significantly narrower than for any other exposure. This study is an example of growth reduction with slight foliar symptoms. Foliar symptoms response most sensitive in early July. | Loblolly pine *Pinus taeda* (2 wks old)<br>Sycamore (1 wk old) Seedlings | Kress and Skelly, 1977 |

APPENDIX (continued)

| TIME | CONCENTRATION | RESPONSE OF VEGETATION | PLANT SPECIES | AUTHOR |
|---|---|---|---|---|
| 10. 6 hours | 0.025 ppm $SO_2$ + 0.05 ppm ozone | 20-28 day-old white pine needles on ramets, exposed 9 am - 3 pm. All sensitive clones adversely affected. Response judged in terms of needle elongation (growth) and foliar lesion or tip necrosis. Author calls response synergistic. 0.025 ppm $SO_2$ or 0.05 ppm = threshold; 0.10 ppm $O_3$ = 20% necrosis. | East. White pine<br>Pinus strobus | Houston, 1974 |
| 11. 22 hours. | 100 to 500 ppm $SO_2$ aqueous sol. | Swelling of thylakoid disks in chloroplasts disintegration of intro-chloroplast membranes in mature needles. Absence of mitochondria. Author state 100 - 500 ppm aqueous $SO_2$ is equivalent to 1000-fold lower concentration in air or 0.1 - 0.5 ppm.<br>Older needles more sensitive. Photosynthesis reduced/measured by evolution of $O_3$. | Lodgepole pine<br>Pinus contorta | Malhotra, 1976 |
| 12. Annual Average of 24-hour measurements | 0.06 - 0.009 ppm | 20 ± 5% loss in radial growth. Premature drop of some needles. Reduction of above-ground plant organs that can lead to important losses of water in catchment areas (at Hamr air monitoring station, Erzgebirge, Germany). At higher $SO_2$ levels (0.026 - 0.037 ppm, annual average) rapid death of individual trees and whole groups of trees (at Brandov air monitoring station, Erzgebirge, Germany). | Fir forests<br>Abies sp. | Materna, 1973 |

APPENDIX (continued)

| | TIME | CONCENTRATION | RESPONSE OF VEGETATION | PLANT SPECIES | AUTHOR |
|---|---|---|---|---|---|
| 13. | Average 30 min. $SO_2$ conc. for period of 1954-1963 | 0.045 ppm | Foliar injury, premature defoliation, reduced photosynthesis, decreased radial and volume growth - 10 cubic feet per acre per year ($1,171,000 in 1963), premature death, rough bark, purple bark, reduced blister rust, small leaf growth, more severe symptoms with high pollution levels. 750 square miles in Sudbury, Ontario. | East. White pine<br>*Pinus strobus* | Linzon, 1971 |
| 14. | 4 - 8 hr/ day, 5 days/wk 4-8 weeks | 0.1 ppm $SO_2$ + 0.1 ppm ozone | 16% needle necrosis (chlorotic, yellow spots, current year needles thin and twisted) shedding of older needles far exceeded damage responses from single exposures. .1 ppm $SO_2$ injured 4% of needle area; .1 ppm $O_3$ injured 3% of area. | East. White pine<br>*Pinus strobus* | Dochinger *et al.*, 1970 |

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## SECTION 5

# INVESTIGATION OF THE IMPACT OF COAL-FIRED POWER PLANT EMISSIONS UPON INSECTS: ENTOMOLOGICAL STUDIES IN THE VICINITY OF COLSTRIP, MONTANA

by

J. J. Bromenshenk

## INTRODUCTION

Although this report concentrates on the entomological investigations in the vicinity of Colstrip, Montana, from June 1, 1975, to March 1, 1977, it also includes a synopsis of earlier reports and, therefore, serves as the major report of the baseline phase (pre-operational) and monitoring phase (post-operational) of the study. The rationale, methodologies, and preliminary results were published in the Second Interim Report (EPA, 1976). Insect studies at the sulfur dioxide treatment plots (ZAPS) are presented in section 14 of this publication, while related fungal and vegetational investigations are elaborated in sections 4 and 13.

The unifying hypothesis of the EPA-funded Coal-Fired Power Plant Project is that methods can be developed to predict the bioenvironmental impacts of coal-fired power plants before any damage occurs. The basic assumption is that air pollutants are major contributors to damage, defined as negative changes in the components and processes of an ecosystem. Our ancillary hypothesis is that methods can be developed to serve this purpose, based on the use of indicator species of plants, fungi, and insects as early warning systems and as continuous monitors of atmospheric pollutants.

The southeastern Montana area was essentially free of anthropogenic pollutants in the ambient air prior to the construction of the Colstrip power plants (U. S. Environmental Protection Agency (EPA), 1976; Montana Department of Health and Environmental Sciences (DHES), 1972; Montana Department of Natural Resources and Conservation (DNRC), 1974; Westinghouse Environmental Systems (WES), 1973). Therefore, it was hypothesized that if we measured, quantified, and mathematically described pollutant-sensitive characteristics of key indigenous species of the flora and fauna of the major ecosystems (cool season-short grass and ponderosa pine-skunkbush) prior to the operation of the Colstrip generating units, we could monitor any observable changes in these characteristics as the ecosystems were subjected to pollutant challenge. Also, by the appropriate analysis of temporal and spatial factors, we hoped to correlate these changes with the level of air pollution and relate them to other ecosystem components.

The uniqueness of this approach lies in the opportunity it presents to participate in a multi-disciplinary, multi-agency study, to conduct baseline studies in a basically uncontaminated area, and to pursue these studies during initial and subsequent impact of emissions from the Colstrip power plants. The use of biological indicators or estimators is not new, and the rationale for their use was stated well by Thomas et al. in a 1974 review of biological indicators of environmental quality:

> A continuous monitor of any environmental variable is superior to periodic sampling because the concentration of pollutants or intensity of stress varies with time. Because organisms integrate their responses through time and because they react to all synergistic and antagonistic effects of combined pollutants or stresses, they do provide convenient full-time monitors. These biological indicators give the actual responses of individual organisms or populations, rather than predict biological responses from physical measurements obtained through instrumentation.

However, it is essential that these indicators be sensitive to perturbations, practical to monitor and measure, and reliable predictors of ecological and economic impacts. To satisfy these criteria and to test the previously stated hypothesis, we proposed and carried out, in progressive order, these study objectives:

(1) Selection and establishment of permanent study sites (ponderosa pine-skunkbush, grassland, and apiary) distributed to maximize gradient exposures to the Colstrip emissions;

(2) compilation of inventories of insect and fungal populations, infestations, and damage to indigenous plant species at the study sites to provide a basis for the selection of more specific groups for use as biological estimators;

(3) analysis and selection of insect and fungal populations with a diversified but understandable interrelationship with indigenous plant species in order to examine effects of air contaminants on symbiotic associations;

(4) selection and testing of disease- and injury-causing fungal and insect species to be utilized for more intensive study;

(5) selection and testing of beneficial fungal and insect species to be utilized for more intensive study;

(6) chemical analysis of the selected indigenous plants, insects, and fungi to establish baseline levels and to discern any accumulation of materials (fluorides and sulfurs) released by the conversion of coal to electricity, and

(7) determinations of physical (pH) and chemical characteristics of precipitation.

After the inventory, selection, and testing phase, baseline data during 1974 and 1975 was gathered to determine:

(1) Growth, health, and pathologies of ponderosa pines, including associated fungal and insect populations and damage parameters;

(2) concentrations of fluorides and sulfurs in the dominant species of indigenous plants of both the ponderosa pine and the grassland ecosystems;

(3) concentrations of these substances in an insect pollinator (honeybees) of grasslands and croplands, and

(4) precipitation chemistry.

In 1976 the precipitation studies were abandoned, the number of pine study plots were reduced and studies were intensified at the $SO_2$ fumigation (ZAPS) plots. The objective was to increase the depth, understanding, and precision of hypotheses regarding those systems, processes, and species which preliminary studies indicated were the components most sensitive or vulnerable to measurable pollutant stress.

## INSECTS

At least 1,400 species of insects inhabit the grassland ecosystems of Montana (personal communication, N. Reese, USDA Rangeland Research Laboratory, Bozeman, Montana). From among these, we elected to study: (1) Pest and beneficial insects associated with ponderosa pine, the dominant tree species in the Fort Union Basin, and a species susceptible to damage by phytotoxic gases and acid rains (EPA, 1973); (2) pollinators, especially honeybees, and (3) ground-dwelling beetles, particularly members of the families Carabidae, Silphidae, and Scarabaeidae (see section on ZAPS). These three sets were chosen because of probable sensitivity to air pollution and ecological and economic importance.

A brief review of the economic considerations follows. Timber resources, composed entirely of one commercial species, ponderosa pine, are harvested from the Custer National Forest and the Northern Cheyenne Reservation. Based on allowable annual cut and timber sale data, these pine stands have a wholesale value of $1,584,000 ($180 per 1,000 board feet) (Gordon, 1975). Harmful insects attack healthy or weakened trees, impairing vitality and causing mortality. Bark beetles, historically, have destroyed more standing timber in western forests than all other insects combined, and defoliators have ranked second as destructive agents (Keen, 1952).

Agriculture is Montana's leading industry, according to annual reports of the Montana Department of Agriculture Statistical Service. In 1973 the cash receipts for the seven-county area of southeastern Montana totaled $110,042,600 or 10 percent of Montana's marketable produce. Pollinating insects are vital to various crops and indigenous plant species. The honeybee is important not only to Montana's agriculture, but also to that of California and other states where 25 percent of Montana's bees are taken in the winter for pollination purposes.

Montana is an important contributor to the nation's honey industry, ranking first in the nation for honey yield per colony and fifth for total yield. Montana honey is premium grade white "clear" honey and is used to upgrade honey from other regions. Approximately eight percent (6,600) of the honeybee colonies of Montana are located in our study area. These colonies produce about 739,200 pounds of honey annually (based on state average yield), with a wholesale value of $332,640 and a retail value of $724,416 (personal communication, W. Kissinger, State Apiarist). But estimates of the economic value of honeybees as pollinators range from 20 to 100 times their wholesale value as honey and wax makers (Winski, 1974; E. McGregor, 1976; F. Moeller, personal communication). Using these figures, it appears that the honeybees of southeastern Montana are worth from $6.6 million to $33 million annually just as pollinators, excluding their value to California growers.

## LITERATURE REVIEW

Insects and other invertebrate animals seldom are studied in air pollution investigations, yet the available literature suggests significant interactions of insects and pollutants. Sulfur oxides, nitrogen oxides, carbon oxides, particulates, hydrocarbons, fly ash, acid mists, and numerous trace elements impact entomological systems (bibliography in Appendix A). For example, Hillmann and Benton (1972) conducted investigations near a 615 MW coal-burning generator in central Pennsylvania and observed several consequences to insect populations which were linked to sulfur dioxide air pollution, while a European study associated honeybee mortalities with arsenic emitted from a coal-fired power plant, 6 km distant (Ferencik, 1961).

Air contaminants may accumulate in the tissues of insects by ingestion, respiration, or penetration through the integument (Debackere, 1972). Toxic substances may be passed along the food chain and accumulate in predatory insects, resulting in host-parasite or host-predator imbalances (Dewey, 1972; Hillmann, 1972; Hay, 1975). In addition, pollinating insects, especially the social insects, appear to be very susceptible to toxicosis from atmospheric pollutants (Dewey, 1972; Knowlton _et al_., 1950; Bromenshenk and Carlson, 1975). Social insects such as domestic and wild bees forage over large distances, transport materials back to the colony and store them as food supplies. These insects are morphologically and behaviorally specialized for the collection and transport of small particles (i.e., pollen) which may lead to the inadvertent accumulation of small, particulate contaminants.

Studies of pesticides and of anesthesia with carbon dioxide and nitrous oxides indicate that the effects of toxic substances on insect physiology, biochemistry, and behavior include sublethal effects, such as disorientation, memory loss, and permanent changes in behavior as well as the more obvious lethal effects (Schricker and Stephens, 1970; Ribbands, 1950). Gerdes _et al_. (1971a and 1971b) found that 1.3 to 2.9 ppm of hydrogen fluoride reduced hatchability and fecundity of fruit flies, _Drosophila melanogaster_, probably as a result of genetic damage, and Ramel (1967) induced chromosome disjunction by feeding _D. melanogaster_ 0.25 mg mercury. Recently, European investigators (Debackere, 1972; Toshkov _et al_., 1974) concluded that some insect species, particularly honeybees, have a very high potential as sensitive and efficient indicators of toxic substances and acidic solutions.

Numerous reports indicate that "trees weakened or injured by pollutants are more likely to be attacked by insects that normally require weakened trees for successful reproduction" (Haegle, 1973). The number of arthropod pests that have been associated with pollution-predisposed trees is small. However, certain sucking insects or those that feed in concealed places, such as under bark, are more likely to proliferate, while foliage-feeding insects are more subject to poisoning because of their greater exposure both to airborne toxins and through feeding (Hay, 1975). Two questions repeatedly are raised in reviews of the subject (Haegle, 1973; Hay, 1975; Ciesla, 1975): Are trees predisposed to arthropod attack and damage because of air pollution stress? Do air pollutants affect the predators and parasites of a pest arthropod and, as such, affect important regulators of pest population numbers? These questions are particularly important in view of the conclusion of Mattson and Addy (1975) that phytophagous insects act as regulators of primary production and nutrient cycling of forests and thus have long-term interactions with fundamental ecosystem processes.

## MATERIALS AND METHODS

The techniques and materials employed for the entomological investigations, including the monitoring and analysis for the accumulation of sulfur and fluoride in insects, are lengthy and have been published previously (EPA, 1976; Gordon _et al_., 1976; Kay _et al_., 1975; Carlson _et al_., 1974). Summaries of these procedures, modifications, deletions, and additions are indicated under the reports on specific objectives.

## OBJECTIVE #1

### SELECTION AND ESTABLISHMENT OF PERMANENT STUDY SITES DISTRIBUTED SO THAT GRADIENT EXPOSURES AND RESPONSES TO AIR POLLUTANTS FROM THE COLSTRIP POWER PLANTS OCCUR

In September of 1976, participants from federal agencies and research institutions convened to examine the role of applied ecology in environmental problem solving (Johnson, 1976). They concluded that post-action impact monitoring programs are potentially valuable aids to the improvement of modeling and predictive capabilities and provide valuable opportunities to measure the results of controlled experimental perturbations of diverse ecosystems. They stressed the need for an experimental design that could identify unforeseen or underestimated impacts, provide a base against which to compare post-action impacts, improve future predictions, and be amenable to information transfer.

We believe that plot selection is a critical element of pre- and post-action impact evaluations. First, any controlled study of perturbations in this type of field investigation depends on plot distribution which in turn should consider known or suspected wind channels from the stationary source and the degree of exposure of the plot to contaminants emitted by the source. For example, because fluorides are transported in the atmosphere, vegetation on ridges or elevations downwind from the emission source is more likely to be subjected to fluoride stress than vegetation upwind from the source or in valleys (Carlson and Dewey, 1971). Second, air pollution impacts may be short-term and acute or long-term and chronic. The latter may be discernible only after a period of several years. Therefore, for maximum usefulness, study plots should be relatively permanent as regards location and accessibility, which is why plots were located on public domain whenever possible.

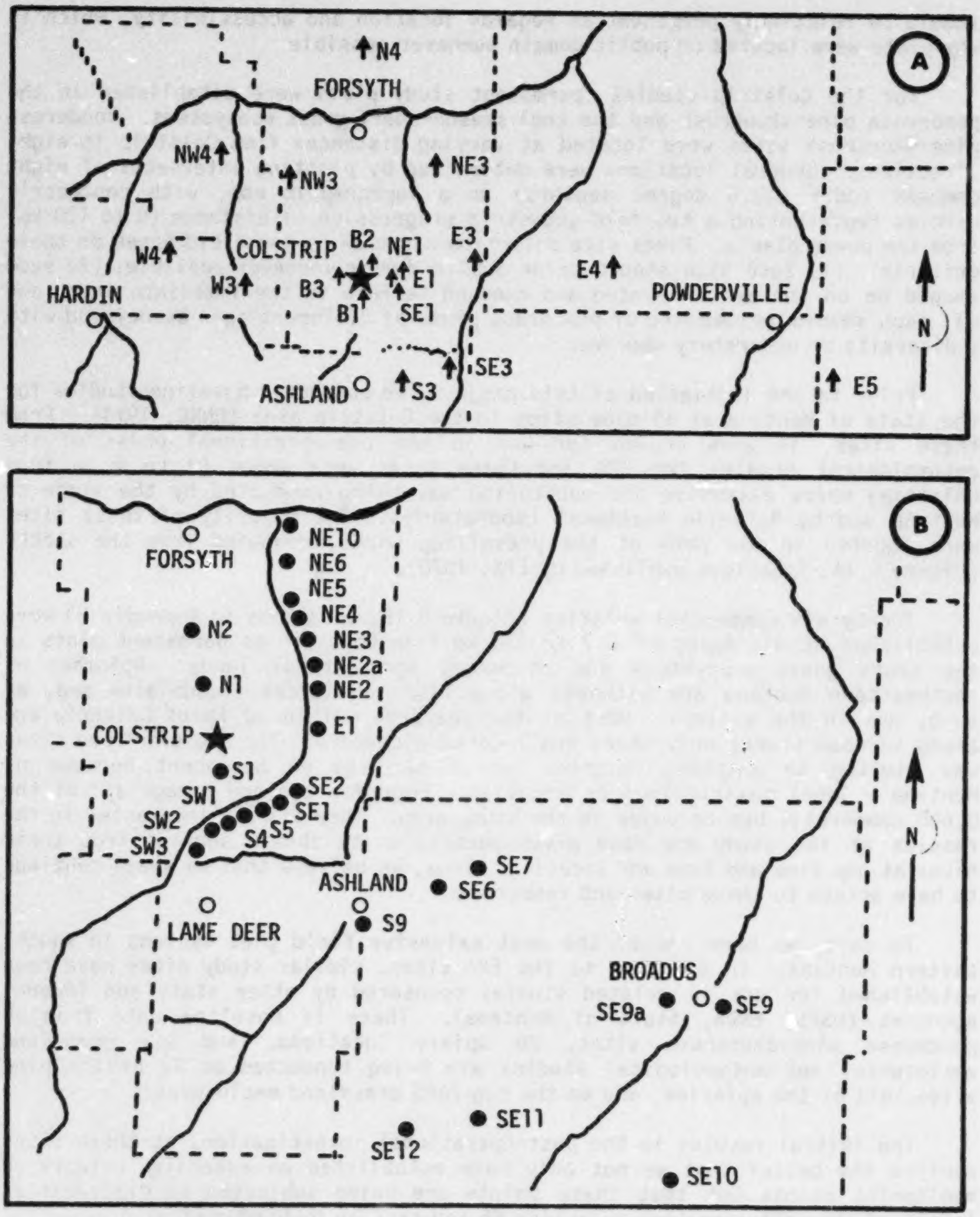
For the Colstrip studies, permanent study plots were established in the ponderosa pine-skunkbush and the cool season-short grass ecosystems. Ponderosa pine-skunkbush sites were located at varying distances from Colstrip in eight directions. General locations were determined by plotting intersects of eight compass radii (45.0 degree sectors) on a topographic map, with concentric circles representing a two-fold geometric progression of distance (8 to 120 km) from the power plants. Final site selection was made in the field based on these criteria: (1) Each site should be on public domain whenever possible; (2) each should be on the most elevated and exposed terrain in the immediate area, and (3) each should be composed of ponderosa pines of different ages associated with a diversity of understory species.

Prior to the initiation of this project, we conducted baseline studies for the state of Montana at 35 pine sites in the Colstrip area (DNRC, 1974). From these sites, 14 were chosen for use in the pre-operational phase of the entomological studies for EPA and three sites were added (3 to 5 km from Colstrip) where extensive air monitoring was being conducted by the state of Montana and by Battelle Northwest Laboratories. The majority of these sites were located in the path of the prevailing winds, downwind from the stacks (Figure 5.1A, locations published by EPA, 1976).

Twenty-six commercial apiaries (Figure 5.1B, locations in Appendix B) were established at distances of 6.7 to 130 km from Colstrip as permanent plots in the short grass ecosystems and bordering agricultural lands. Apiaries of southeastern Montana are situated along river and creek floodplains and, as such, are in the valleys. Most of the beeyards within 20 km of Colstrip are along Rosebud Creek; only three are located elsewhere. The choice of bee sites was limited to existing apiaries, which tend to be permanent because of Montana's legal restrictions on location. Four men own and manage all of the 6,600 commercial bee colonies in the study area. They all are interested in the results of the study and have given permission to obtain samples from their hives at any time and from any location. Thus, we believe that we shall continue to have access to these sites and resources.

To date, we have one of the most extensive field plot systems in southeastern Montana. In addition to the EPA sites, similar study sites have been established for use in related studies sponsored by other state and federal agencies (USFS, ERDA, State of Montana). There is baseline data from 50 ponderosa pine-skunkbush sites, 26 apiary locations, and six grassland enclosures, and entomological studies are being conducted at 17 of the pine sites, all of the apiaries, and on the two ZAPS grassland exclosures.

The initial results in the post-operational investigations at these sites confirm the belief that we not only have established an extensive network of monitoring points but that these points are being subjected to differential levels of air pollution (see Objective #5 and section by Gordon *et al.*).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POWDERVILLE
B1
SE1
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SE3
ASHLAND
S3
E5
B
NE10
FORSYTH
NE6
NE5
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N2
NE3
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COLSTRIP
S1
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S4
SW3
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SE7
ASHLAND
SE6
S9
LAME DEER
BROADUS
SE9a
SE9
SE11
SE12
SE10

Figure 5.1. Colstrip study area; locations of (A) ponderosa pine-skunkbush and (B) apiary sampling sites.

## OBJECTIVE #2

## COMPILING INVENTORIES OF INSECT POPULATIONS, INFESTATIONS, AND DAMAGE TO INDIGENOUS PLANT SPECIES AT THE STUDY SITES TO PROVIDE A BASIS FOR THE SELECTION OF MORE SPECIFIC GROUPS FOR USE AS INDICATOR SPECIES

These surveys were initiated at the ponderosa pine-skunkbush sites to determine the insect fauna present, to determine the status of insect pests of ponderosa pine and their potential for outbreak, and to determine the status of beneficial insects, such as insects predacious on foliage feeders. It was hypothesized that this information would provide a basis for the selection of more specific groups of insects as indicator species, as well as provide supplemental information, such as habitat information and distribution records. We did not intend to expend large amounts of study resources amassing extensive species lists or collecting quantitative data such as estimates of absolute population size or diversity indices. It was felt that the natural variability of many insect populations precluded or greatly reduced the probability of using this type of data for meaningful post-action evaluations of pollution-induced perturbations or of separating these perturbations from the interaction of other physical and biotic variables.

Investigators from the Colorado Natural Resources Ecology Laboratory (CNREL) attempted to document changes in total arthropod population numbers and biomass on grassland sites using techniques that had successfully shown responses of arthropods to artificially-induced environmental stresses such as moisture and grazing pressure. They encountered problems applying this approach at the EPA $SO_2$ fumigation plots because of low population numbers and high natural variability. However, their extensive insect sampling provided species inventories for the grassland ecosystems; these inventories allowed us to concentrate our attention on the pine ecosystems.

Several techniques were used to conduct insect inventories: (1) Examination of state and federal entomological collections (State Department of Forestry and USDA Forest Service, Region 1) for identified species collected from southeastern Montana timber stands; (2) searches of records and collections pertaining to insects and insect outbreaks in ponderosa pines of eastern Montana; (3) on-site observations and sampling procedures (Appendix C) conducted in August and October, 1974, and once every two or three weeks from May 1 through October 15, 1975; (4) examination of insects in or on foliage samples obtained from all of the ponderosa pine sites, both from the EPA studies and those from the Forest Service project, and (5) utilization of sticky traps (after Williams, 1973) which consisted of 35 x 45 cm rectangles of 1.5 ml aluminum coated with Tack-Trap® and fastened 3.5 meters above the ground to the trunks of each of five trees at five locations. The sticky traps were changed every six weeks from May 5 through November 20, 1976. The purpose of this trapping, begun in 1976, was to trace the phenology of some of the major or dominant insects on pines and, hopefully, to obtain specimens of ephemeral adult forms, which were necessary for the identification of a few species which we had seen only in immature stages. Results from the sticky traps were not complete at the time (March 1, 1977) of preparation of this report.

All insect specimens were labeled according to site, date of collection, and indexed by number in a log containing comments. The specimens were preserved in 80 percent ethanol in glass vials until they could be identified.

The emphasis for this part of the entomological investigations has been to provide supportive data for the ponderosa growth-health studies (see section by Gordon et al. for study plan and results). The growth-health studies quantify pathologies occurring on pine foliage obtained from ten permanently marked trees at each of the plots. Foliage samples were taken from upper and lower crown positions on the side of the trees facing Colstrip. The foliage was then subjected to analysis of sulfur and fluoride content, and the percent occurrence of ten needle pathologies (e.g., insect, fungal, pollution damage), the percent needle retention, needle length and cross-sectional area, and chlorophyll content. Pathology determinations were made for each group of 100 needles from the preceding four years of growth. The lengthy procedures have been published (Gordon et al., 1976); procedures and results are summarized in the section by Gordon et al. of this report. These procedures, including sample size and data handling, were evaluated by PEDCO Environmental Specialists, Cincinnati, Ohio, and their recommendations regarding the study plan have been incorporated.

An insect checklist is included in Appendix D. On-site measurements and evaluations dealing with tree physiology and insect-damaged portions of trees other than foliage are summarized in Appendix E. The most frequent and serious insect damage syndromes are illustrated in Figures 5.2 and 5.3.

From our pre-operational studies, the following conclusions were drawn regarding the status of insect species present at the ponderosa pine-skunkbush plots:

(1) Compared to most stands of ponderosa pine in Montana, trees on the study plots in southeastern Montana were exceptionally healthy and generally demonstrated little or almost no insect, disease, or pollution damage, except in a few localized areas.

(2) The primary causes of tree mortality appeared to be porcupine girdling, storm breakage and blow-down, and fire. Insects and disease as primary causes of death were rare, except on the Cheyenne Indian Reservation which has had a history of occasional insect outbreaks in timber stands and continues to have some localized insect problems.

(3) Insect populations were generally low and insect damage was minimal.

(4) The most prevalent destructive insects and damage included: (a) Pine cone boring by the larvae of beetles and moths (Conophthorus, Laspeyresia, and Dioryctria spp.), which occurs on all plots and occasionally destroys significant numbers of cones and seeds; (b) scale insects (Matsucoccus secretus Morrison) found on most trees under the fascicular sheaths or between the needles in two year or older needles; (c) foliage feeding by several weevils such as Scythropus elegans (Couper) observed on all plots and Magdalis sp. observed on new growth at several plots; (d) pine loopers (Phaeoura mexicanaria (Grote)) seen at six plots but in very low numbers; (e) several sawflies (Neodiprion spp.) seen feeding on foliage at several plots but only on

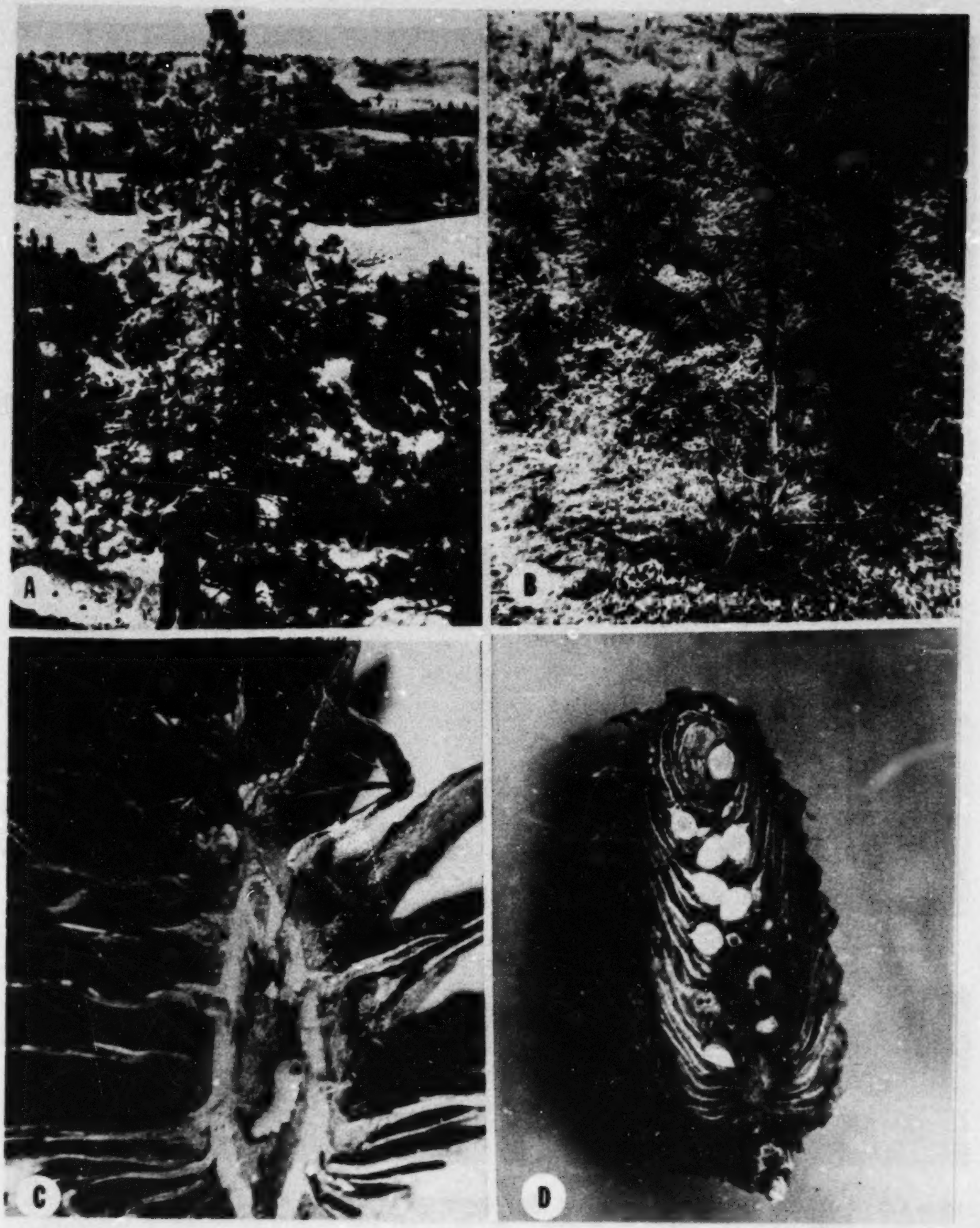


Figure 5.2. A. Ponderosa pine killed by bark beetles (Dendroctonus ponderosae). B. Young ponderosa pine girdled by a porcupine, which will cause the tree to die. C. Immature pine cone of ponderosa pine attacked by Conophthorus ponderosae Hopk. The cone is dwarfed by the attack of the insect. D. A pine seed moth larva, probably Laspryresia piperana (Kearf.), working through the pith into seeds. The larval stage of three species of pine seed moths are indistinguishable.

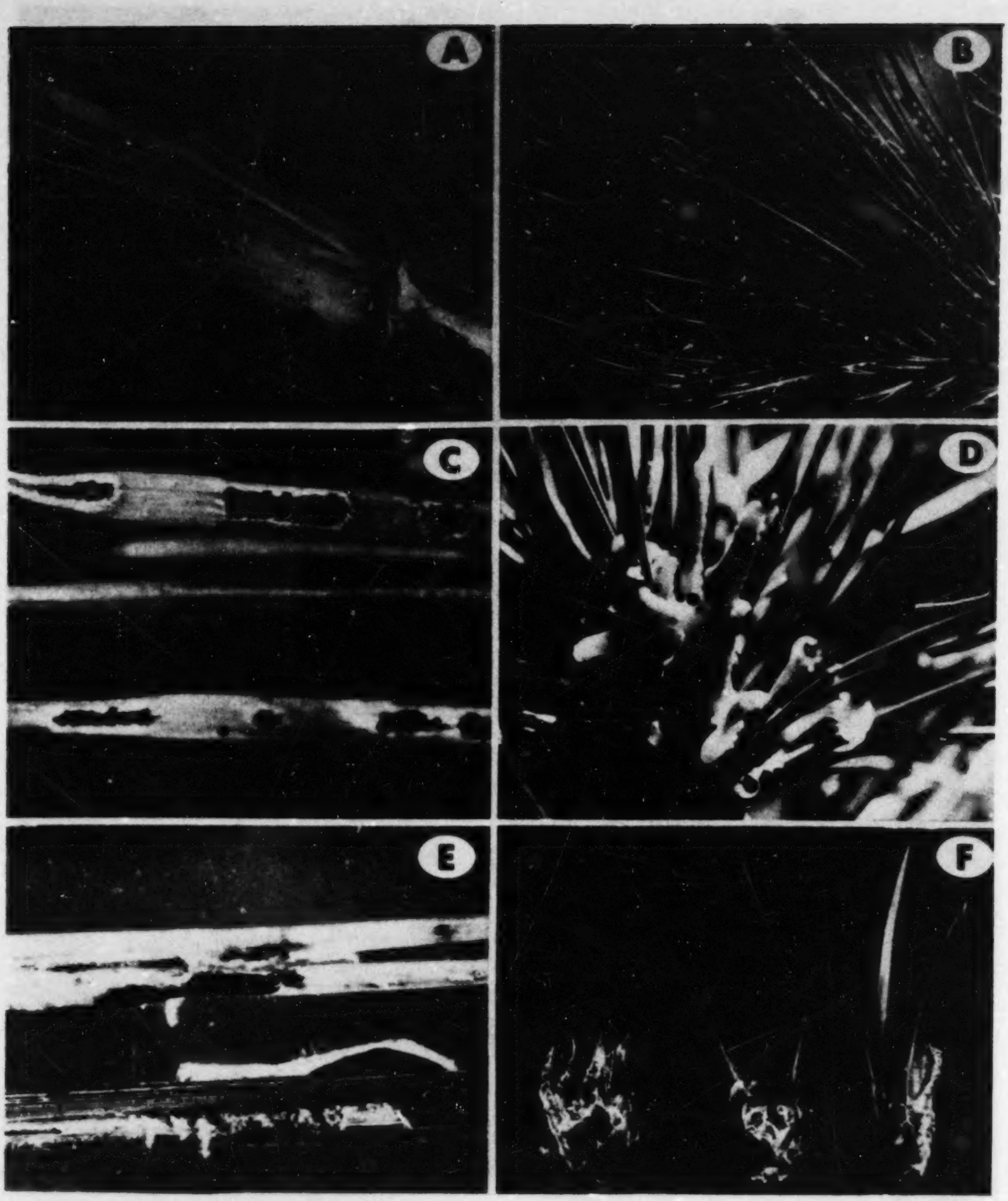


Figure 5.3. A. Basal portion of a needle of Pinus ponderosa showing the necrotic symptom of damage found under the fascicular sheath. B. An infestation of pine needle scales (Phenacaspis pinifoliae) on ponderosa pine. C. Notches chewed in the needles of Pinus ponderosa by weevils such as those of the genus Scythropus. D. A cluster of larvae of a species of Neodiprion (sawfly) feeding on needles of Pinus ponderosa. E. Close-up of needles of Pinus ponderosa damaged by a pine needle miner. A larva is inside the needle. F. Shortened needles with bulbous bases characteristic of attack by several pine gall midges, family Cecidomyiidae. Many of the western species have not yet been named.

occasional branches; (f) infrequent, localized infestations of bark beetles (*Dendroctonus ponderosae* Hopk. and *Dendroctonus brevicomi* LeC.) and engraver beetles (*Ips calligraphus* (Germar) and *I. pini* (Say)), and (g) ubiquitous populations of aphids.

(5) The most prevalent insects during the summer months were lady bird beetles (particularly *Cleis* sp. and *Neomysia* sp.) and a leaf-footed plant bug (*Leptoglossus* sp.), which was common in the autumn months. The beetles are predacious and are reported to feed on a variety of foliage feeders such as mites, scale insects, and aphids.

(6) Many other insect predators, parasites, and parasitoids prey on the harmful insect species. Particularly conspicuous beneficial insects were lacewings of the family Chrysopidae and Mantispidae, several Icheumonids (parasitic Hymenoptera), and beetles of the families Cleridae and Coccinellidae. In addition, a profusion of dead aphids exhibiting large exit holes in the abdominal region indicated the interaction of an unidentified but prevalent parasite.

(7) The potential for an immediate epidemic of destructive insects was considered low except in a few localized areas. The pine looper, *Phaeoura mexicanaria*, occurred in epidemic numbers on the Northern Cheyenne Reservation and the Custer National Forest in 1969 and 1970 (Dewey, 1970). About 20,000 acres were heavily defoliated and another 43,000 were moderately to lightly defoliated. The defoliation appeared to predispose many trees to attack by bark beetles (*Ips calligraphus*, *I. pini*, and *Dendroctonus valens* LeC.). A collapse of the pine looper population in 1970 was attributed to the spread of a pathogenic bacterium.

M. McGregor, leader of the Bark Beetle Evaluation and Control Group, USFS, Region 1, inspected several Colstrip pine sites during August 3-8, 1975, and his report (in Gordon *et al.*, 1976) closely agreed with our own observations. McGregor concluded that a weakening of stands might lead to another population build-up of the pine looper (*P. mexicanaria*) but discerned no immediate potential for outbreak. He noted infestations of the mountain pine beetle (*D. ponderosae*) and the pine engraver beetle (*I. pini*) in a blow-down at one plot in the Custer National Forest and in areas of thinning of second-year growth of ponderosa pine in the Three-Mile Creek area, which had a potential for a localized build-up by 1976.

Besides providing species lists and information on the pre-operational status of insects associated with ponderosa pines, these insect surveys may be extremely useful for post-impact monitoring. Rather than attempting to directly quantify insect population dynamics, we are monitoring the insect damage incurred by foliage by assessing the percent needles attacked or consumed by insects. In his book on ecological methods as applied to insect populations, Southwood (1975) considered estimates of the amount of a plant consumed, expressed either as a quantity of material consumed or as some index of damage, to be the most meaningful biological measure of the effect of an insect population on a plant stand, although these estimates are only an approximate index of the size of the insect population. Damage per se is of more intrinsic interest to us than absolute insect numbers because insect damage affects growth rate and is reflected by growth rings (Mott et al., 1957) and because increased insect damage to conifers has often been associated with pollution-stressed trees (review by Haegle, 1973). However, in every case, reports of tree mortality or decreased production due to insect attack on pollution-stressed trees relied on studies conducted after the stationary source(s) had been in existence for some period of time. Generally, there was little, if any, documentation of insect conditions prior to the operation of the source. Thus, if an insect outbreak occurred, the argument often was made that the outbreak was a natural part of the life cycle of the pest and was a random or coincidental occurrence rather than the result of increased levels of a toxic pollutant.

In the Colstrip area, we quantified foliage damage caused by several agents including insects (see section by Gordon et al.) prior to the operation of the power plants, determined the current status of insects known to be destructive to ponderosa pine, and obtained records concerning the history of insect problems in the area on the nearby commercially usable stands of timber, the Custer National Forest and the Indian reservations. We consider the area to be free of serious insect problems, as evidenced both by records and by observations of the tree stands at each site. However, some insect species were identified which appeared to be of particular interest to observed foliage damage and post-impact responses. These are discussed under Objectives #3 and #4.

## OBJECTIVE #3

### ANALYSIS AND SELECTION OF INSECT POPULATIONS WHICH HAVE A DIVERSIFIED BUT UNDERSTANDABLE INTERRELATIONSHIP WITH INDIGENOUS PLANT SPECIES AT THE STUDY SITES IN ORDER TO EXAMINE THE EFFECTS OF AIR CONTAMINANTS ON SYMBIOTIC OR COEVOLVED SYSTEMS

Inventories (USDA Rangeland Laboratory, Bozeman, Montana; USFS, Region 1, Missoula, Montana; Environmental Studies Laboratory, University of Montana, Missoula) of the pine and grassland ecosystems of southeastern Montana indicated that a minimum of 1,500 to 2,000 species of insects commonly are associated with the indigenous plant species. We elected to concentrate our efforts on a few specific life systems, an approach which is believed to yield good quality control and the opportunity to test feasible hypotheses. A "life system" is defined as the part of an ecosystem which determines the existence, abundance, and evolution of a particular population. According to this concept, the inherited properties (array of genotypes) of a population transform environmental resources of matter and energy into phenotypes or the individuals of the species. These individuals form populations with group characteristics (e.g., birth rates, dispersal, mortality). The continuance and abundance of a population are determined by interactions between inherited characteristics of the subject and essential attributes of its environment. The intrinsic qualities of the subject species and of its environment are termed the co-determinants of population numbers. The co-determinants control population performance (e.g., reproductive capacity, mortality, immigration, and emigration or, in other words, numbers and persistence). The manifestations of determinant processes are certain ecological events, including primary demographic events such as matings, birth rates, mortalities, densities, and distributions and secondary events which affect or modify the primary occurrences such as resource availability and inimical agents, both biotic and abiotic. This life system approach to the ecology of insect populations was formulated and presented by Clark et al., 1967.

Having decided to pursue a life system approach, a list of 28 insect species (EPA, 1976) was drawn which were thought to be: (1) Major components of the pine and grassland ecosystems of the study area; (2) dominant and/or useful indicators or estimators of pollution; (3) economically important, and (4) closely coevolved with dominant, indigenous plant systems. The rationale for our emphasis on the latter point was expressed in a recent symposium on the coevolution of plants and animals (Gilbert and Raven, 1973). They stated in the introduction:

> Coevolutionary relationships are, by definition, the product of historical change (=evolution); yet this historical change is still proceeding. The relationships between elements in a contemporary ecosystem are dynamic in both an evolutionary and in an ecological sense, and depending upon the generation times involved, may even change very rapidly during a given period of observation. This is all the more likely in these times of extensive environmental pollution and the consequent alteration of relationships within ecosystems that must affect all of our observations.
>
> Tightly structured organism-organism relationships hold considerable promise for the understanding of the area as a whole.

To test the hypothesis that tightly structured insect/plant interactions may change rapidly as a consequence of pollution-induced alterations of these relationships within ecosystems, we selected from the list of 28 species a few herbivorous insects of ponderosa pines (Objective #4), insect pollinators and the plants from which they obtain their nectar and pollen (Objective #5), and ground-dwelling beetles which act as scavengers and decomposers and return nutrients to the soil ecosystems, which in turn affects plant growth.

## OBJECTIVE #4

## SELECTION AND TESTING OF INJURY- AND DISEASE-CAUSING INSECTS TO BE UTILIZED FOR MORE INTENSIVE STUDY

The original objective was to select and pre-test, for use at the study sites, insect/plant systems which not only appeared to be of greatest interest to project goals but also would be easy to manipulate. Towards this goal, we reared (EPA, 1976) laboratory populations of grasshoppers (Melanoplus bivittatus) and several herbivorous pine insects such as the pine needle scale, (Phenacaspis pinifoliae (Fitch)), bark beetles (Ips and Dendroctonus spp.), and aphids (Cinara spp.). In general, the insects were provided with their preferred food such as boughs of foliage for needle feeders, bolts of wood for bark beetles, and native grasses for grasshoppers. Work was terminated with grasshoppers because of the interest and subsequent work with these insects by the investigators from Colorado (CNREL) and also because five years of personal experience with the grasshopper systems of Montana's rangelands revealed that the population dynamics and host relationships of these organisms were extremely complex and that population changes were unpredictable. In addition, a study by Dewey (1972) indicated that grasshoppers near an aluminum smelter accumulated less toxic materials (fluoride) than pollinators, predators, or many other foliage feeders.

Inventories, examinations, and measurements of foliage pathologies revealed the presence of a scale insect (identified as Matsucoccus secretus Morrison) found under the fascicular sheath of needles, occurring often on or near a zone of basal necrosis (Figure 5.4) in foliage generally at least two years or older. It is not known at present if this insect is a primary factor, a secondary invader, or a coincidental occupant in the production of all or a portion of this necrosis. The basal necrosis is similar to that which Dr. Gordon has produced in the Environmental Studies (EVST) greenhouse by "acid mists." Therefore, it is felt that it is essential to clarify the relationship of this insect to the necrosis and that the implications of this relationship are far reaching. Also, Siewniak (1971) reports that Matsucoccus pini occurs most frequently on Scotch pine in areas with polluted air. For these reasons, highest priority is being given to a study of the lifecycle and relations of this insect to ponderosa pine.

This insect first was observed in 1974 but was seen only occasionally. Examination of foliage samples in autumn of 1975 indicated that the insect was more common than it was thought, probably because, as Keen (1952) states, they are extremely difficult to detect because they push their way under the fascicular sheath and may take on the color of their surroundings.

A specialist, Dr. Douglas R. Miller (USDA Agricultural Research Service, Beltsville, Maryland), verified the identity of the insect. The insects are small, less than 1.5 mm long, and inconspicuous. The sheath must be dissected from the needle in order to find the insect; if the sheath is pulled away from the needle, the insect is removed with the sheath.

Macroscopic and microscopic examination of needle bases disclosed that the basal necrosis which occurs beneath or just above the fascicular sheath appears to grade, in degree of damage, from a pale or dark brown blemish on the surface of the needles to a small localized enlargement (blister) of the mesophyll tissues; this pushes the hypodermal and epidermal tissues out into a blister. An eruption or splitting of the epidermal and hypodermal tissues may occur, usually in association with the blistering. The original hypothesis, which has since been discarded, was that there were two distinct needle tissue disease syndromes; the first was termed basal necrosis for the purpose of needle pathologies, the second was termed basal scale. The reason for the latter terminology was that cases were noticed in which several insects, especially the basal scale, were near or in the areas of tissue eruption or hypertrophy. Based on continued histological and macroscopic examinations of the insect and of this disease, it is now believed that there is no real difference between these damage syndromes except of degree.

An approach towards obtaining an understanding and a working knowledge of the basal scale insect involves several procedures. In order to determine the extent of the distribution of the insect, our laboratory staff has been recording the presence or absence of the insect and the life stage of the insect

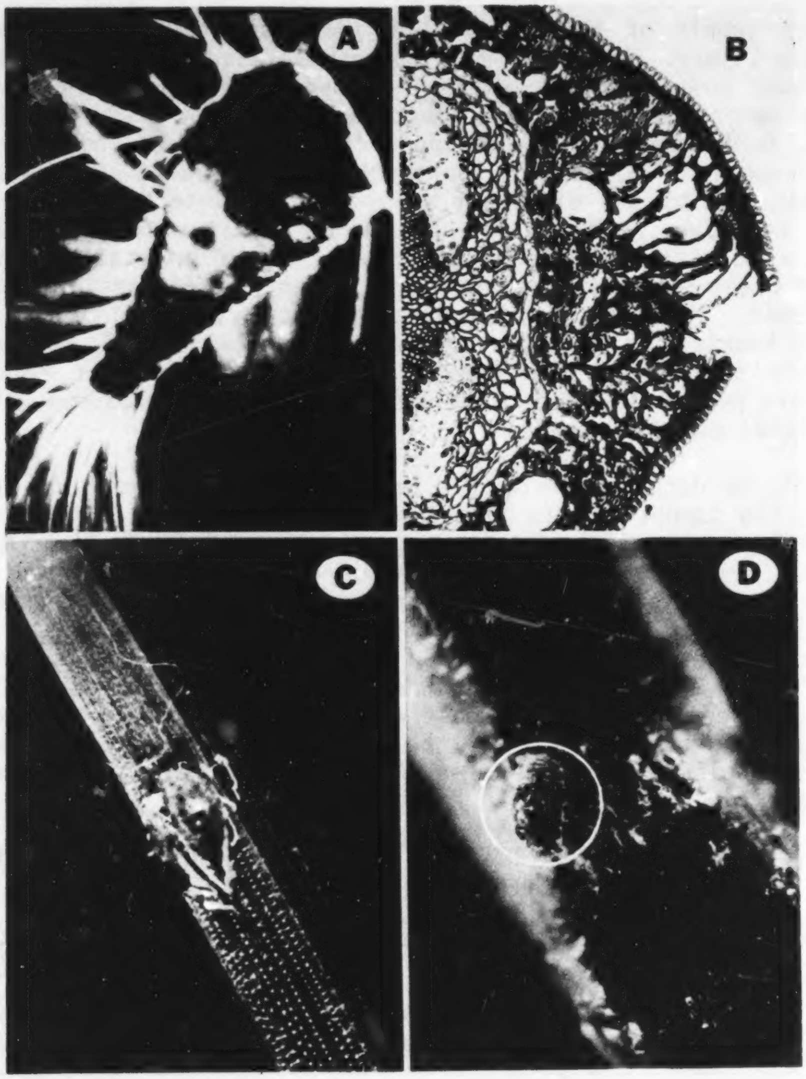


Figure 5.4. Scale insect (Matsucoccus secretus) and the hypertrophied or "blistered" needle base which is vulnerable to attack by insects and saprophytic fungi. The fascicular sheaths of the needles (Pinus ponderosa) have been removed.

A. Dark-field photomicrograph of an unidentified insect found under the fasicular sheath. B. Cross-section of erupted needle. C. Scales in permanent feeding position on a needle base. D. Motile stage of scale, or possibly of an adelgid, in temporary feeding position near erupted tissues of a needle.

insect on each sample of 100 needles/tree/plot used to quantify the various types of needle injury. Examination of the needle bases was facilitated by the use of a 70-power zoom objective binocular dissecting scope (Bausch and Lomb). In 1976 there was a search for this insect at all of the plots for all of the projects (EPA, ERDA, USFS) or on a minimum of 500,000 needles from 22 plots from mid-March through mid-September. Representative specimens of the different pathology types and of the different stages of the insect were prepared for histological analyses, killed and fixed in FAA(formalin-aceto-alcohol), sectioned by a paraffin technique (Johansen, 1940), and stained either with Fuelgin's Fast Green Schedule or a modified Fleming's triple satin method (Raske and Hodson, 1964). The modified Fleming triple stain was used to differentiate insect feeding tracts; using this procedure, xylem stains blue, phloem orange, and mucoprotein secretions from feeding red. Observations of sections and photographs were performed using a Reichert "Zeptopan" microscope with bright field, dark field, and phase optics.

The results to date concerning the prevalence of the insect are summarized in Table 5.1. The insect appears to be common in the southeastern Montana area, including Billings, occurs in relatively low numbers, and seems to prefer younger trees. Macroscopic and microscopic examination indicated that the motile adults and larvae frequently were entrapped by exudates from erupted needle tissues and that feeding often occurred adjacent or proximal to the areas of basal necrosis, but not necessarily in the damage zone. In fact, most of the larvae in permanent feeding positions were on healthy-appearing parts of the needle. This, in conjunction with observations by Dr. Gordon that the basal damage probably occurs before the meristematic tissue is fully differentiated, indicates that the insect may not cause the damage but may be attracted to the area of tissue damage.

TABLE 5.1. PERCENT OCCURRENCE OF TOTAL BASAL NEEDLE NECROSIS AND OF BASAL SCALE INSECTS ON NEEDLES OF PONDEROSA PINE AT SIX SITES NEAR COLSTRIP AND BILLINGS, MONTANA - 1976.

| Year of Foliage | Number of Foliage Samples (trees) | Number of Needles Dissected | Percent Non-necrotic Needle Bases | Percent Necrotic Needle Bases | Percent Basal Insects |
|---|---|---|---|---|---|
| 75 | 48 | 2611 | > 98 | < 2 | < .5 |
| 74 | 48 | 2742 | 81.6 | 18.4 | 4.9 |
| 73 | 46 | 2514 | 50.8 | 49.2 | 7.5 |
| 72 | 10 | 461 | 34.7 | 65.3 | 13.9 |

A review of the literature concerning Matsucoccus (bibliography, Appendix F) revealed that some species have caused severe damage to pines over extensive areas and that some forms cause needle galls; but the damage syndrome discerned in this study is not characteristic of any reported damage attributed to Matsucoccus. Histological comparisons of the feeding patterns of these organisms in relation to feeding and damage by other insects (Figures 5.5 and 5.6), fungi, and abiotic factors such as frost, drought, and acid mist should clarify the role of this insect. There are Matsucoccus infested trees in a greenhouse at Missoula and these are being used as a source of basal scale for further studies of life cycles, feeding, and damage. We hope to establish a suitable population for inoculation tests based on Koch's Postulates.

## OBJECTIVE #5

### SELECTION AND TESTING OF BENEFICIAL INSECTS TO BE UTILIZED FOR MORE INTENSIVE STUDY

The previous objective emphasized coevolved systems of insects and plants in which the insects were detrimental both in terms of harm to the plant and in subsequent economic loss because of plant damage. However, there are many coevolved insect/plant systems in which the insect is beneficial. The most familiar aspect of advantageous insect-plant interaction is the pollinator relationship. Honeybees were chosen as the subject species because of their known sensitivity to pollution stress (Toshkov et al., 1973), ecologic and economic importance, availability (in terms of numbers), and manageability. It is believed that they will be indicative of the population responses of other pollinators, and to test this hypothesis honeybees and other pollinators are being observed at the $SO_2$ fumigation plots for comparative purposes. The native bees, especially the physically smaller species, may be much more susceptible to zootoxins than honeybees (Johansen, 1972).

We would like to work with predatory and predacious insects that attack forest pests and with the predatory ants of the grasslands; but the initial pretesting indicated that this exceeded study resources, necessitating priority assignments. In 1976, studies of ground-dwelling beetles were added at the ZAPS plots on the basis of previous surveys and biomass studies by the Colorado investigators (CNREL).

While behavioral responses of honeybees to controlled exposures of $SO_2$ have been investigated at ZAPS, the studies near Colstrip have concentrated on the use of honeybees (Apis mellifera L.) as accumulators and indicators of the extent and magnitude of the phytotoxic and zootoxic effluents from the Colstrip power plants (see Objective #6).

## OBJECTIVE #6

### CHEMICAL ANALYSIS TO DETERMINE SULFUR AND FLUORIDE CONTENT OF INDIGENOUS PLANTS AND INSECTS TO ESTABLISH BASELINE LEVELS AND TO MONITOR ANY ACCUMULATION OF THESE MATERIALS WHICH ARE RELEASED BY THE CONVERSION OF COAL TO ELECTRICITY

As mentioned previously, it was hypothesized that honeybees would be useful in the collection and detection of toxic materials from the environment. Numerous reports supported this hypothesis, although many were based on studies in Europe during the 1950's (review by Debackere, 1972). The usefulness of the published information from these studies is sometimes questionable because of two problems. One is the lack of a standard expressing the concentrations of

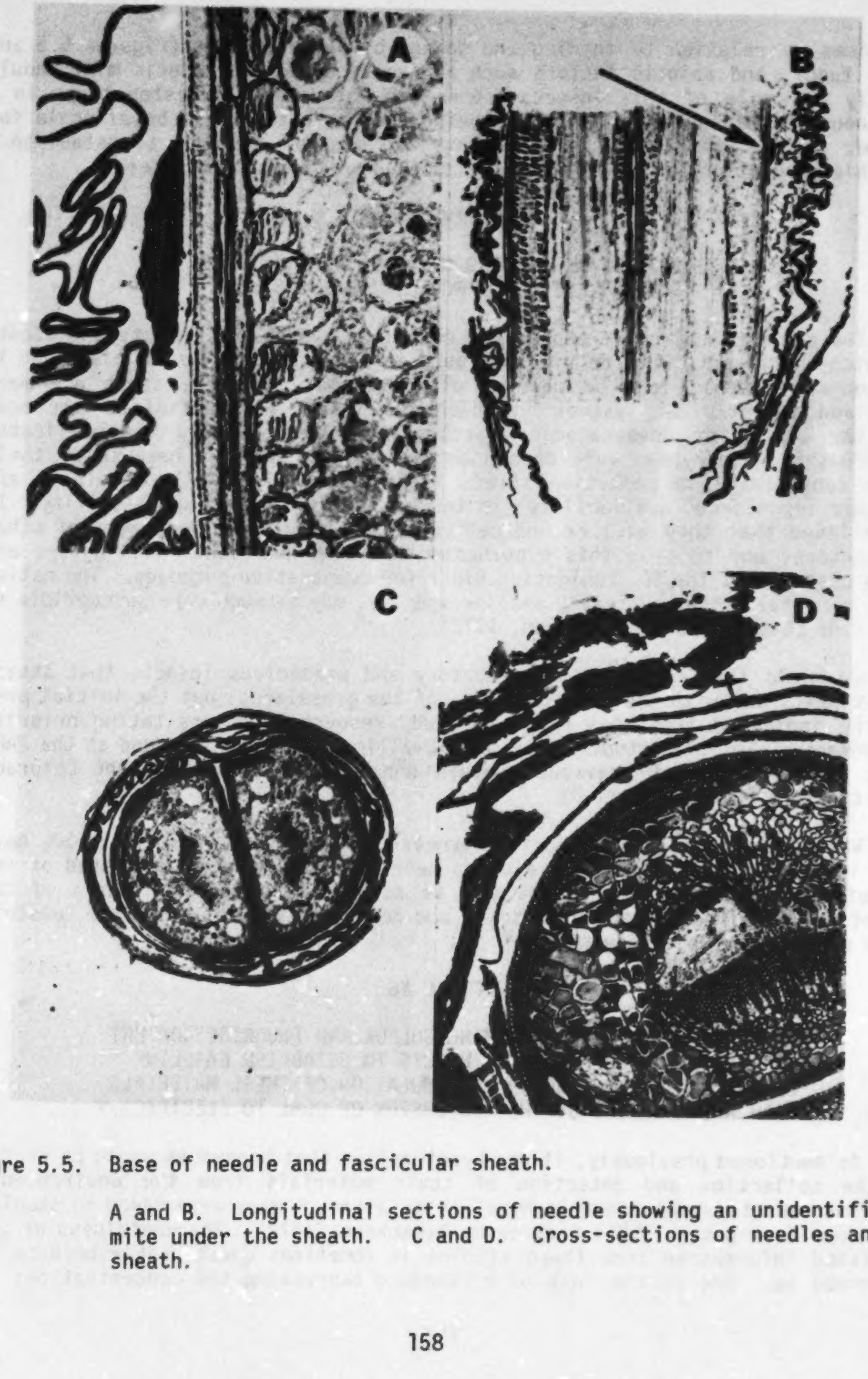


Figure 5.5. Base of needle and fascicular sheath.

A and B. Longitudinal sections of needle showing an unidentified mite under the sheath. C and D. Cross-sections of needles and sheath.

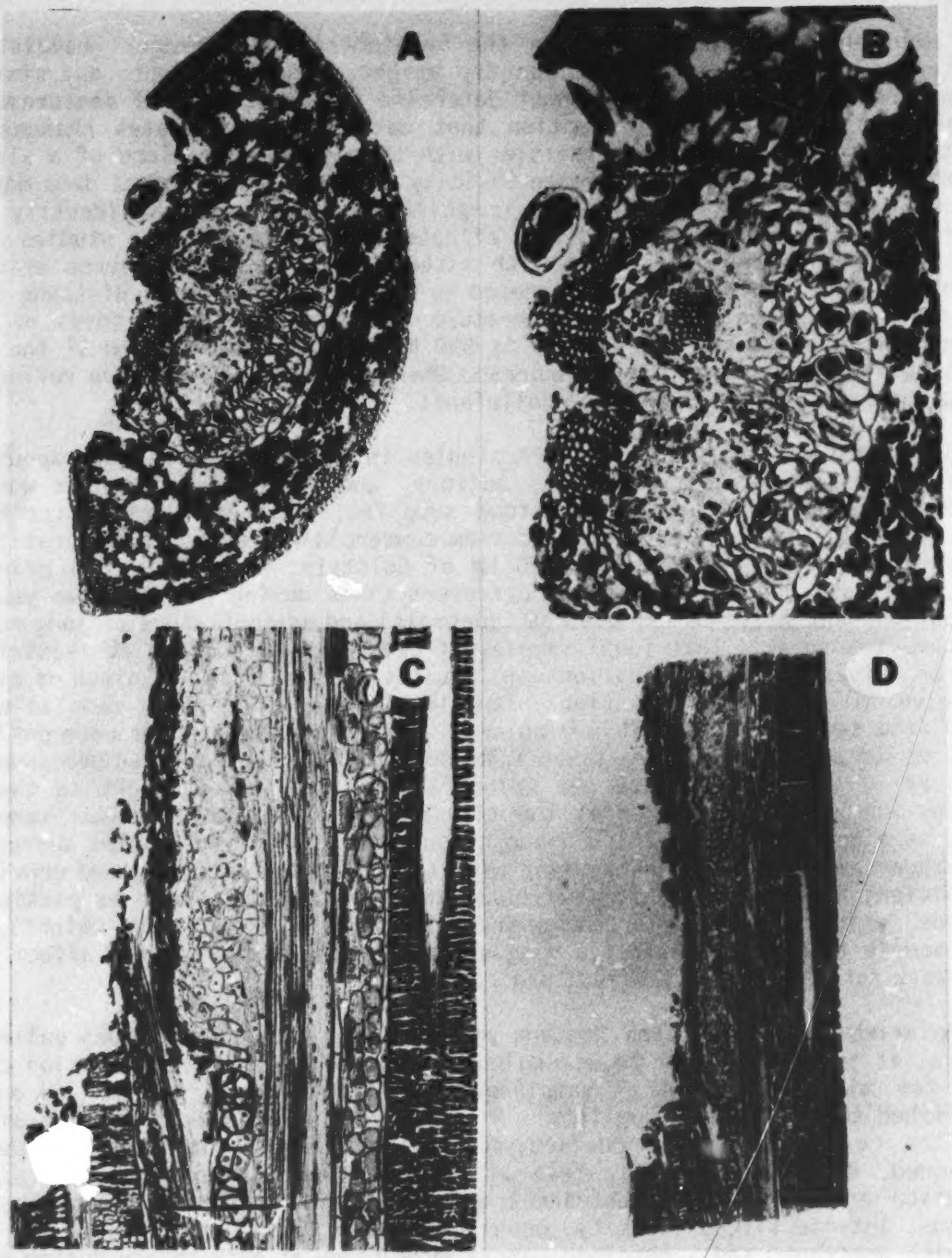


Figure 5.6. Weevil damage to needles of *Pinus ponderosa*.

A and B. Cross-sections of damaged needles and insect. C and D. Longitudinal sections of weevil damaged needles.

toxic substances in insects and in the vegetation they forage. Levels are reported as g/insect, g/kg, mg%, ppm dry weight, ppm wet weight, and similar designations so vague that one cannot determine the basic unit of measurement. The other problem is the assumption that data which correlates changes in insect populations, such as mortalities with tissue concentrations of a single contaminant, may be used to estimate toxicity or harm. This type of data may be sufficient to demonstrate harm by air pollutants but does not identify the specific agent and ignores synergistic effects. In addition, these studies were conducted near industrial sources with sites distant from the source used as controls. These controls were designated as such because of their distance from the source. But the acid rain literature indicates that some forms of air contaminants may impact areas as far as 500 km from the source, and if the air pollution from the source was widespread, these controls may not have reflected pre-operational baseline levels of pollutants.

Since the initiation of the EPA studies in August 1974, we have acquired quantitative analyses of fluorides, sulfurs, and pesticides in adult worker honeybees, their pollen and honey food supplies, and their water supplies. Bees, honey, and pollen were obtained from commercial apiaries at 26 locations. Twelve of the apiaries are within 20 km of Colstrip, are utilized as primary sites, and have been sampled eight different times during the last two years. The most distant beeyards are used as (controls) and as indicators of long range pollutant transport. Individual samples of bees were taken from at least four different hives, and a combination sample was collected from a minimum of eight to twelve hives at each location. Individual honey and pollen samples were pooled from several cells within a colony. Individual bee samples were defined as 200 to 300 adult worker bees taken from the same colony. Combined bee samples consisted of 150 to 300 bees per colony taken from each of eight to twelve colonies and grouped together at the time of collection. Individual samples served as a measure of variation among colonies; the combined samples served as an average sample, allowed comparison with the individual samples, and provided a sufficient number of bees for those analytical procedures, such as pesticide analyses, which require a large number of bees (100 g or more wet weight). A 100 g sample of bees taken from a single colony could significantly affect the field work force and as such affect production.

Apiaries in southeastern Montana are laid out in rows of wooden pallets, each pallet supporting four to six colonies (Figure 5.7A). The selection criteria for hives consisted of sampling all suitable hives in each quadrant established by the rows of pallets. A suitable hive was one in good physical condition (e.g., paint not peeling, supers and covers intact and properly positioned, clean appearance), free of obstructions and contaminants (e.g., vegetation and dirt at the entrance) and containing a strong colony (e.g., vigorous, intense flight activity, populous). Four individual bee samples, one from each quadrant, and a combined sample were taken at each apiary during each sample period in 1975 and 1976, excluding the spring periods.

An acrylic plastic vacuum apparatus (Figure 5.7B) was developed for collecting bees at the entrance to a hive to minimize inadvertent contamination at the time of collection. All samples for sulfur and fluoride analysis were frozen immediately by bagging them in plastic Whirl Paks® and placing them under a block of dry ice in a styrofoam cooler. Since pesticide determinations may be

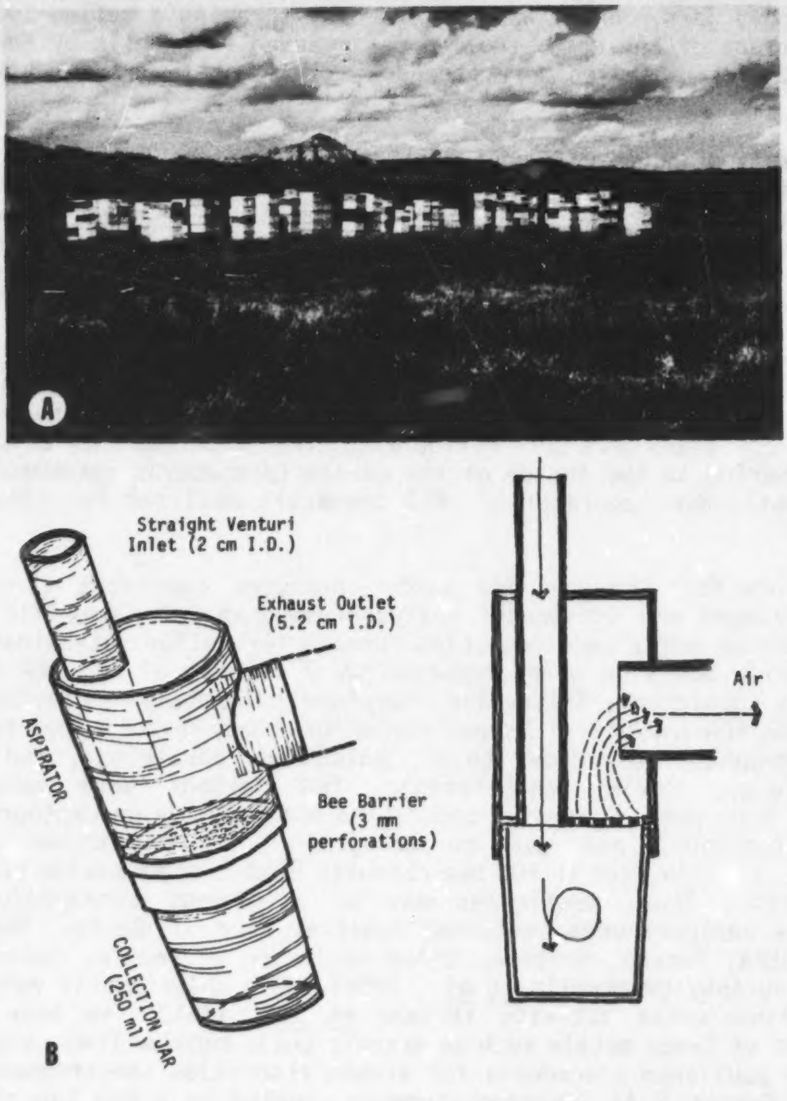

A
Straight Venturi Inlet (2 cm I.D.)
Exhaust Outlet (5.2 cm I.D.)
ASPIRATOR
Air
Bee Barrier (3 mm perforations)
COLLECTION JAR (250 ml)
B

Figure 5.7. A. Apiary at SW-3 by Rosebud Creek near Colstrip, Montana. B. Acrylic aspirator used to obtain honeybees for chemical analyses. A battery (12 volt) powered squirrel cage fan provides a vacuum and is connected to the exhaust outlet via a flexible plastic hose. Air and bees are drawn through the venturi inlet; the bees are deposited in the collection jar. The jar is removed by unscrewing it from the aspirator, giving a sharp downward rap to force the bees to the bottom, and quickly removing and capping the jar.

confounded by residues from plastics, we used a metal vacuum collector, based on the design of the acrylic apparatus, and stored the frozen samples in new one-pound glass honey jars. Honey was collected by scraping a teflon-coated spoon across the surface of the comb. Pollen was removed from cells by the use of a narrow plastic pick. Samples were stored at -10°C until they could be prepared for analysis.

Clean sampling and storage equipment was of the highest priority. Cleaning procedures were determined by the analyses to be performed. All plastic equipment, except the clean, sterile Whirl Paks®, was cleaned by washing in detergent and tap water, rinsing with 1:1 nitric acid, then with tap water, followed by a one normal rinse with hydrochloric acid, then with tap water, and a final rinse with distilled and deionized water (Taras et al., 1971). The equipment was air-dried, face or mouth downward, on a plastic pad. Glass and metal equipment was cleaned by washing in detergent and tap water, rinsing with distilled and deionized water, and then rinsing with acetone followed by a rinse with hexane. Bottles were air-dried, mouth downward, on an aluminum foil sheet. The mouths of all glass jars were sealed with clean aluminum foil to exclude any impurities adhering to the inside of the covers (procedures recommended by the Beltsville Pesticides Laboratory). All chemicals utilized for cleaning were reagent grade.

Preparation for fluoride and sulfur analyses consisted of drying and grinding whole bees and subsequent analyses using an Orion specific ion electrode for fluoride and a Leco induction furnace for sulfur determination (EPA, 1976). Pesticide analyses were conducted by R. Thomas of the EPA Biological Investigations Laboratory, Beltsville, Maryland. The analytical methodology is outlined in the FDA Analytical Manual for chlorinated insecticides (e.g., DDT, DDE, DDD), phosphate pesticides (e.g., malathion, parathion), and carbamate pesticides (e.g., sevin, carbofuran). FDA methods were sensitive to approximately 0.01 ppm. Screening procedures utilized gas chromatography, thin layer chromatography, and gas chromatography in conjunction with mass spectrometry. In 1975, 700 to 800 bee colonies died from pesticide poisoning in the study area. Thus, pesticides may be a serious confounding factor. Cholinesterase analyses were performed courtesy of R. J. Barker, Bee Research Laboratory, USDA, Tucson, Arizona, using isolation procedures based on thin-layer chromatography (Winterlin et al., 1968) and a chlorimetric determination of acetylcholinesterase activity (Ellman et al., 1961). We have initiated determinations of trace metals such as arsenic (As), cadmium (Cd), and lead (Pb) in bees using published procedures for atomic absorption spectrophotometry. A Unicam SP90A Series 2 AA Spectrophotometer coupled to a Van Lab strip chart recorder was utilized. The results will be published in the next interim report.

Unless indicated, all statistical evaluations were performed on a Hewlett Packard 65 calculator or a Dec-10 computer and utilized standard statistical programs, including mean, standard deviation, standard error, F ratios based on variances, and Bartlett's test for homogeneity of variances. Significant differences (.05 confidence level) of variances allowed the use of a t-test for equality of means with variances unequal, while non-significant differences of variance allowed the use of a t-test for the equality of means with equal variances (F ratios and t-tests from Sokal and Rohlf, 1969). All confidence levels were computed for the 95 percent lower and upper confidence limits of the mean.

Quality assurance tests of the analytical procedures utilized recovery efficiency studies, and a reciprocal exchange with other laboratories for a comparison study was carried out by the Boyce Thompson Institute. The procedures are precise, rapid, and show very good agreement with other methods. The fluoride probe, based on several years of data, is accurate to ± 0.10 ppm, while the Leco method for sulfur in bee tissues is accurate to ± 4 percent. The accuracy of the honeybee analyses and the pollen analyses is very good; however, the samples must be well mixed after grinding. We did not experience the difficulties reported by Nation *et al.* (1971) who encountered difficulties in obtaining a homogenous sample of *whole* bees for the atomic absorption analysis of major and trace elements in bees, pollen, and royal jelly. Their procedures were similar to ours except that they ground their bees in a mortar while we used a Wiley mill which produces a finely ground powder which will pass through a 40 mesh screen. Nation *et al.* concluded that the very hard structures of the head, antennae, wings, and legs made it virtually impossible to get a homogenous sample but thought that finely ground materials or analyses of specific body regions might improve recovery efficiency.

For consistency, all levels of fluorides and sulfurs presented here are given on the basis of ppm dry weight (μg/g dry weight). To facilitate comparisons to published reports of levels of contaminants in bees, which are often expressed as a unit weight per one bee rather than per unit of mass or weight of bee tissue, we present the results of a series of experiments conducted to determine the weights of honeybees and the moisture content of bee tissues (Table 5.2).

The results from the preliminary samples in 1974 are given in Table 5.3. The mean fluoride content of adult worker bees from 11 sites was 7.4 ppm, while the mean sulfur content was 4,390 ppm. Carlson and Dewey (1971) reported that 10.5 ppm $F^-$ was characteristic of bees from a clean or control area in western Montana, while bees near an aluminum smelter contained 221 ppm $F^-$. Obviously the mean 7.4 ppm $F^-$ indicated a clean area. In addition, European investigators have reported levels from 0 to 40 ppm as characteristic of bees from clean areas (Lillie, 1970). We found only one report of total sulfurs in bees. Hillman (1972) fumigated colonies with 3.65 ppm $SO_2$ for nine weeks and found no significant differences in sulfur levels between fumigated bees and non-fumigated adult or larval bees. He reported levels of 3,200 to 4,600 ppm in adult bees and 1,800 to 3,900 in larval bees.

Our interest in honeybees extended beyond their potential as collectors of contaminants from the environment. We also wished to discover whether the levels of these contaminants would increase in bees in the Colstrip area to levels damaging to the bees and to the bee industry in terms of effects such as reduced numbers, strength, and vigor, thereby decreasing production. In order to test the ability to detect contaminants such as fluoride in bees, beekeepers in Anaconda, Billings, Butte, and Columbia Falls were asked to send samples of bees from areas known to be subjected to high ambient air concentrations of pollutants and/or areas in which beekeepers had repeatedly experienced bee poisonings or unexplained (e.g., pesticides, disease) losses.

TABLE 5.2. WET WEIGHTS, DRY WEIGHTS, AND MOISTURE CONTENT ADULT WORKER HONEYBEES WITH STANDARD DEVIATIONS.

| G Wet Weight/Bee | G Dry Weight/Bee | Percent Moisture |
|---|---|---|
| 0.092 ± 0.004* | 0.031 ± 0.002 | 66.85 ± 1.58 |
| 0.088 ± 0.006† | 0.028 ± 0.002 | 68.66 ± 1.51 |
| - ‡ | - | 66.53 ± 1.95 |

*Bees collected from ten different apiaries in September 1974 near Colstrip, Montana. Each value is a mean of ten independent samples of 500 bees. Each sample of 500 bees represents bees from eight colonies in the same apiary, 5,000 bees total.

†Bees collected from 14 different apiaries in August 1976 near Colstrip, Montana. Each value is a mean of 15 independent samples of 142-216 bees. Each sample of bees from a separate colony, 2,661 bees total.

‡Bees collected from 15 apiaries in July and August 1976 near Colstrip, Montana. Percent moisture based on samples from 64 separate colonies, approximately 250 bees/sample; 16,000 bees total.

TABLE 5.3. FLUORIDE AND SULFUR LEVELS IN ADULT WORKER HONEYBEES IN PPM DRY WEIGHT, WITH STANDARD DEVIATIONS*

| Site | ppm $F^-$ | ppm S |
|---|---|---|
| BN-2 | 5.2 ± 0.57 | 4600 ± 282 |
| BNE-5 | 7.2 ± 0.85 | 4700 ± 141 |
| BNE-4 | 5.5 ± 0.00 | 3900 ± 141 |
| BNE-3 | 15.7 ± 0.21 | 4300 ± 141 |
| BE-1 | 6.0 ± 0.64 | 4300 ± 141 |
| BSE-1 | 9.1 ± 1.48 | 4700 ± 141 |
| BS-1 | 5.1 ± 0.14 | 4600 ± 282 |
| BS-4 | 11.0 ± 0.28 | 4600 ± 0.00 |
| BS-5 | 6.1 ± 0.21 | 4300 ± 141 |
| BSE-2 | 7.0 ± 0.35 | 4400 ± 0.00 |
| BSW-1 | 5.2 ± 0.35 | 4000 ± 0.00 |
| Total for All Sites | 7.5 ± 3.21 | 4390 ± 299 |

*Bees collected near Colstrip, Montana in September of 1974. Each value is the mean of two independent sub-samples from a combined sample taken from eight to twelve colonies.

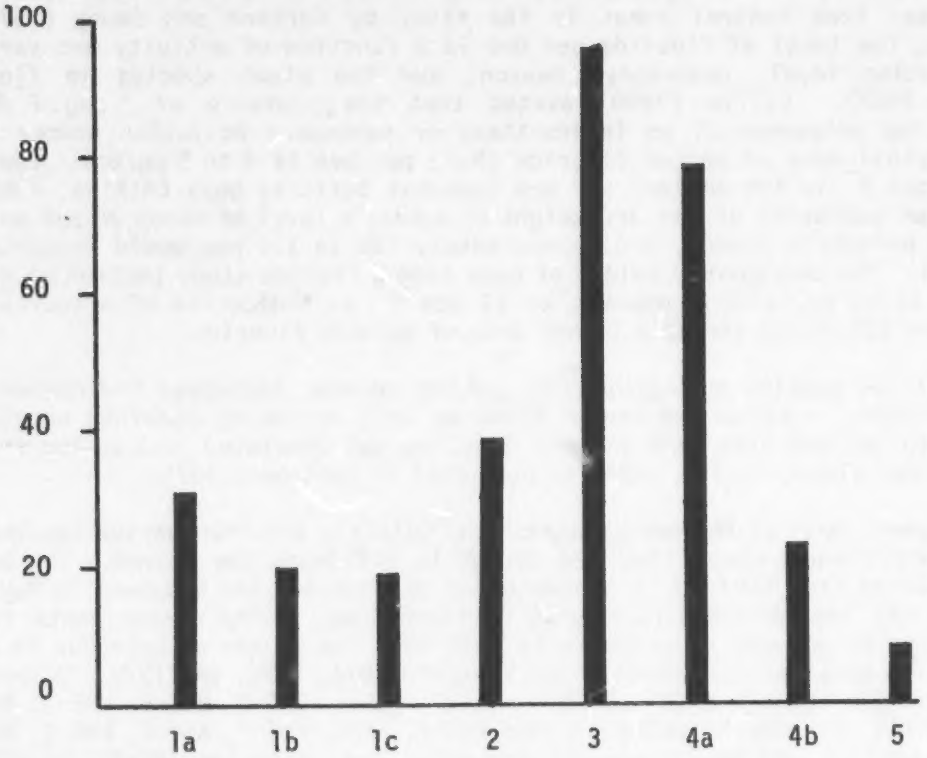
The results are presented in Figure 5.8. It is evident that bees from these areas had substantially higher fluoride levels than the bees near Colstrip and the bees from control areas in the study by Carlson and Dewey (1971). Reportedly, the level of fluoride per bee is a function of activity and varies with pollution level, geography, season, and the plant species in flower (Guilhon, 1962). Lillie (1970) stated that the presence of 1 μg $F^-$/bee indicates the existence of an intermittent or permanent pollution source and that the lethal dose of sodium fluoride (NaF) per bee is 4 to 5 μg/bee. Levels of 4 to 5 ppb $F^-$ in the ambient air are somewhat toxic to bees (Atkins, 1966). Based on our estimates of the dry weight of a bee, a level of about 34 ppm would indicate a pollution source, and approximately 135 to 170 ppm would indicate a toxic level. The average dry weight of bees from a Florida study (Nation *et al.*, 1971) was 32.63 mg, which computes to 33 ppm $F^-$ as indicative of a source of fluoride and 123 to 153 ppm as a lethal dose of soluble fluoride.

In 1976 we sampled throughout the growing season, increased the number of sample locations, sampled individual hives as well as taking combined samples, and began to collect honey and pollen. Sampling was completed just as the first Colstrip power plant, Unit 1, began to burn coal in September 1975.

Each year, most of the bee colonies near Colstrip are transported to almond groves in California where they are rented to pollinate the groves. The bees are transported from Montana in September or October and are returned in May or June. Because bees brought back from California may carry contaminants from that area, colonies were arranged to be left over the winter at apiaries in the primary study area (within 20 km of Colstrip in 1974, 1975, and 1976). Colonies were left at six locations in the fall of 1974 (BS-1, BSE-2, BNE-2, BNE-3, BNE-4, and BNE-10), at nine locations in 1975 (BS-1, BS-4, BSE-1, BSE-2, BNE-2, BNE-3, BNE-4, BNE-10, and BN-1), and at one additional location (BS-5) in 1976. Honeybee colonies in the area southeast of Ashland were never moved from the region and as such served as further controls.

The presence of pesticides and/or pollutants in migratory bees is a potential problem. However, the bees are returned with a minimum of food stores and are in brood boxes. The supers, frames, and other equipment are not taken to California. If contaminants are carried back from California, they should decrease as food stores, equipment, and honeybees are resupplied. The studies indicated that this was the case (Table 5.4). Comparison of fluoride levels in bees over-wintered in the Colstrip vicinity compared to fluoride levels in bees brought back to these sites from California demonstrated no significant difference in fluoride content. All samples in this comparison were collected after mid-June, by which time contaminants carried from California should have been eliminated.

In 1975 it was found that one beeyard (BNE-10) had high levels of fluoride (20 to 108 ppm) in samples from all four collection dates. This same pattern was repeated in 1976. Prior to 1976, the bees at BNE-10 had not been taken to California, and the hives had been at that location for at least three years. Because these colonies had dwindled and were very weak by fall, they were taken to California in the fall of 1975 and replaced the next spring by strong colonies; i.e., the apiculturist's breeding stock. As in 1975, the BNE-10 colonies built slowly and produced very little honey in an area that appeared to



| | |
|---|---|
| 1a,b,c | Butte-Anaconda, Montana; near Stauffer Chemical and Anaconda Smelter |
| 2 | Billings, Montana; near Corette Power Plant |
| 3 | Rosebud, Montana; fluoride in water supply |
| 4a | Columbia Falls, Montana; south of aluminum reduction facility |
| 4b | Columbia Falls, Montana; west of aluminum reduction facility |
| 5 | Colstrip, Montana; from 25 apiaries in Southeastern Montana prior to power plant operation |

Figure 5.8. Mean ppm fluoride in adult worker honeybees from smelter, industrial, and urban areas.

TABLE 5.4. FLUORIDE CONTENT OF BEES OVER-WINTERED IN THE COLSTRIP AREA COMPARED TO BEES TRANSPORTED TO CALIFORNIA AND BACK INTO THE AREA.

|  | PPM |  |
|---|---|---|
| Site | $\bar{x}$ $F^-$ Over-wintered | $\bar{x}$ $F^-$ Transported |
| BNE-4 | 8.9 | 8.5 |
| BNE-3 | 8.1 | 10.0 |
| BNE-2 | 10.2 | 8.2 |
| BSE-2 | 6.8 | 7.4 |
| BS-1 | 9.0 | 6.5 |
| $\bar{x}$ total | 8.4 | 8.3 |
| SD | 2.73 | 2.24 |
| SE | .68 | .60 |
| N | 16 | 14 |

have all the characteristics of an excellent source of honey and pollen. By August 1976, the bees at this location contained 74 to 108 ppm fluoride, the highest fluoride level of any of the apiaries in the study area.

Because we are interested in the total amount of pollutants in or on a bee, we usually do not wash the samples prior to analyses. To test whether fluoride occurred inside or on the outside of the bodies of the bees, combined samples were taken from 18 sites and split before drying. One sample from each pair was dried and ground as per normal procedure; the other was rinsed with distilled and deionized water until the water ran clear. A t-test for the difference in means for each pair ($t_{[16]} = 1.53$) was not significant ($p > .05$), except in a sample from BNE-10 in which the fluoride content was decreased from 60.7 to 27.6 ppm by rinsing. The average fluoride in bees from each of the paired samples from the other 17 sites did not exceed 12 ppm. Low concentrations of fluoride did not easily wash off or out of the bee tissue, whereas at higher levels at least part of the fluoride did so.

Table 5.5 presents the distribution of fluoride in the three divisions of the body. Expressed as concentration of fluoride in the body or per body region, there was no significant difference ($p > .05$) in the amount of fluoride in body regions of the bees demonstrating elevated fluoride levels. Bees with relatively low fluoride content contained the least fluoride (ppm) in the abdomen region. However, the absolute quantity of fluoride (in μg/ body region) in all bees was the greatest in the thorax, which had the greatest mass.

TABLE 5.5. FLUORIDE CONTENT AND WEIGHTS OF DIFFERENT BODY DIVISIONS (HEAD, THORAX WITH LEGS AND WINGS, ABDOMEN) OF ADULT WORKER HONEYBEES. (Each value is the mean of four sub-samples from a combined sample taken from 8 - 12 hives.)

| Body Division | $F^-$ Concentration (ppm) | | | Mean Dry Weight (mg) | Site |
|---|---|---|---|---|---|
| | N | $\bar{x}$ | SD | | |
| Whole Bees | 196 | 97.9 | 1.41 | 26.29 | NE-10 |
| Head | 527 | 97.7 | 1.3 | 5.71 | NE-10 |
| Thorax | 527 | 97.1 | 1.03 | 16.52 | NE-10 |
| Abdomen | 527 | 97.6 | 0.5 | 8.39 | NE-10 |
| Whole Bees | 204 | 11.4 | 0.6 | 26.49 | S-4 |
| Head | 898 | 10.3 | 2.2 | 4.29 | S-4 |
| Thorax | 898 | 12.8 | 0.5 | 14.02 | S-4 |
| Abdomen | 898 | 7.6 | 0.72 | 7.19 | S-4 |

During 1975 bees were collected at different dates throughout the season and analyzed for fluorides. The accumulation of fluoride recorded according to date of collection was not statistically significant (Table 5.6).

Recent (within 24 hours) dead or dying bees collected in August 1975 from traps or from the front of hives contained 8.0 ppm $F^-$, SD = 2.24, SE = .60, N = 14. Bees, from these same hives, that had been dead several days or even weeks and had decomposed or dried from exposure to the weather contained 4 ppm $F^-$, SD = 3.51, SE = 1.42, N = 6, indicating that fluoride was fairly stable even in decomposed or dried tissue.

The fluoride content of bees, pollen, and vegetation collected near Fort Howes in the autumn of 1975 indicated that fluoride may accumulate or concentrate when transferred from plants to pollen to bees (Table 5.7).

In August of 1975 and August of 1976, 114 samples of pollen and bees from the same hives were collected. The fluoride level in bees was four (1975) to six (1976) times greater than the fluoride in pollen. European investigators have reported that contaminants are brought into the hive primarily with the pollen (Debackere, 1973; Toshkov et al., 1974). To test whether the fluoride in bees correlated with the fluoride level in the pollen brought into their hives, Pearson's product-moment correlation coefficient was utilized. The results are given in Appendix G, Tables G-1, G-2, and G-3. However, fluoride in bees did not correlate with fluoride in pollen, and the level of fluoride in pollen was the same for both years.

TABLE 5.6. PPM FLUORIDE IN BEES COLLECTED ON DIFFERENT DATES IN 1974 AND 1975; SAMPLES FROM EACH SITE COMBINED AT TIME OF COLLECTION.

|  | October 1974 | May 1975 | June 1975 | July-August 1975 | August-September 1975 |
|---|---|---|---|---|---|
| $\bar{x}$ | 7.5 | 9.3 | 8.7 | 7.1 | 9.5 |
| SD | 3.09 | 2.3 | 1.08 | 1.77 | 2.38 |
| SE | .63 | .77 | .44 | .42 | .56 |
| N | 24 | 10 | 22 | 18 | 18 |
| No. Sites | 12 | 9 | 17 | 13 | 18 |

TABLE 5.7. PPM FLUORIDE CONCENTRATIONS IN HONEYBEES, POLLEN, AND BLOSSOMS.

|  | Honeybees | Pollen | Sweet Clover | Alfalfa | Snakeweed | All Vegetation |
|---|---|---|---|---|---|---|
| $\bar{x}$ | 9.13 | 1.3 | .7 | .4 | 1.3 | .8 |
| SD | 1.74 | .34 | .56 | .44 | .14 | .61 |
| SE | 1.01 | .17 | .23 | .18 | .10 | .16 |

Efforts were concentrated on late-season collections of bees and bee materials because during late August or early September: (1) Bee colonies were at their peak in size; (2) most of the honey supplies had been gathered and stored; (3) pollen supplies were at a maximum as egg laying and brood rearing declined in response to diminishing supplies of nectar; (4) the alfalfa seed crop had been pollinated; (5) colonies had had a sufficient period of exposure to any environmental contaminants in the region, and (6) there had been several turnovers in bee populations and supplies so that any contaminants brought in from California were likely to be eliminated or greatly diluted. Because the efforts were concentrated on ZAPS in 1976, all of the outlying yards did not get sampled, as had been done in 1975. The results of the fluoride analyses for bees and pollen collected in August of 1975 and 1976 are presented in Appendix H.

Collecting bees before mid-June proved to be impractical because some colonies had been returned from California, others had not, and those that were in the area were often weak, reducing the number of bees which could be sampled without risking harm to the colony. In May of 1975, there were only ten colonies in the Colstrip area from which to take samples, but the average fluoride level of 9.2 ppm was consistent with fluoride levels at these same sites later in the season (Table 5.6). The most distant site, near Biddle, Montana, had higher fluoride in the bees in the spring than later in the season (30.2 ppm, May; 7.9 ppm, August). Needle pathologies typical of pollution damage were observed and increased sulfurs and fluorides were measured in pines approximately .6 km from this yard, indicating a nearby pollution source. The Biddle site is less than 8 km from oil fields which have occasional burn-offs that may impact the nearby apiary and pine locations. Also, bees are medicated in the spring, and each bee colony consists mainly of over-wintered or older bees, all of which could account for higher levels of fluoride.

The high fluoride level in bees at BNE-10 was surprising, and the fluoride in the bees did not correlate with the level of fluoride in pollen. There was no known industrial source of fluoride nearby, and other possible sources such as automobile emissions or the adjacent railroad were encountered at other locations, some of which had very low fluoride baselines. The water supply of the bees was hypothesized as a potential source of fluoride. The Yellowstone River, a slough approximately 20 m away, and a stock tank fed by a constantly flowing artesian spring (the source of the water in the slough) were readily available water sources. The fluoride content of these waters in May of 1976 were, respectively, 0.4 ppm, 11.8 ppm, and 7.0 ppm. Well and surface waters in the vicinity of Colstrip (1 to 20 km) contained 0.0 to 0.6 ppm, with a mean of approximately 0.2 ppm (unpublished, Bureau of Mines and Geology, personal communication, R. Hedges). In August 1976, the waters were sampled where bees were seen drinking at all of the apiaries near Colstrip. Most of the bees obtained their water from Rosebud Creek which had a fluoride level of 0.6 ppm at the majority of the sites. Mean fluoride in bees versus mean fluoride in water for each site had a correlation coefficient of 0.99658 (Figure 5.9), which is significant ($p < .001$). However, this high correlation may result from grouping data from a site with a high level of fluoride in bees and water with data from all other sites at the base of the regression line, with no intermediate values. Bees from an apiary 2.5 km from the Corette power plant at Billings averaged 39 ppm $F^-$, and water from the river near that site averages about 1 ppm $F^-$, according to Montana State Department of Health records. Water appears to affect fluoride in bees, and we shall attempt to refine this observation in 1977 by obtaining bees and water from areas where the level of fluoride in bees falls between the extremes of these specific cases.

The bees at BNE-10, which is near the town of Rosebud, Montana, averaged 7 to 12 times higher fluoride than any other site in the study area; while the water had 16 to 20 times the fluoride as water at other sites. These levels of fluoride in these bees were indicative of a pollution source and were just slightly less than the level of soluble fluoride forms reported to be toxic to bees. The level of fluoride in bees at all other sites was indicative of a clean area (Lillie, 1970) and as such served as a baseline value. Statistically, the results of Bartlett's test of homogeneity of variances and appropriate t-tests for all collection dates indicated that the Rosebud bees were significantly different ($t_{[1111]} = 9.91$, $p < .001$) in terms of fluoride content from bees from all other sample sites in southeastern Montana. The mean value of fluoride in bees at Rosebud fell outside of two standard deviations of the mean for all sites. Therefore, the fluoride data for the bees, the existence of a fluoride source (water), and literature citations indicated that this site was not representative of a baseline level and as such was not directly comparable to the other sites. For these reasons, the data from Rosebud has been excluded in treatment of the data from the study area sites.

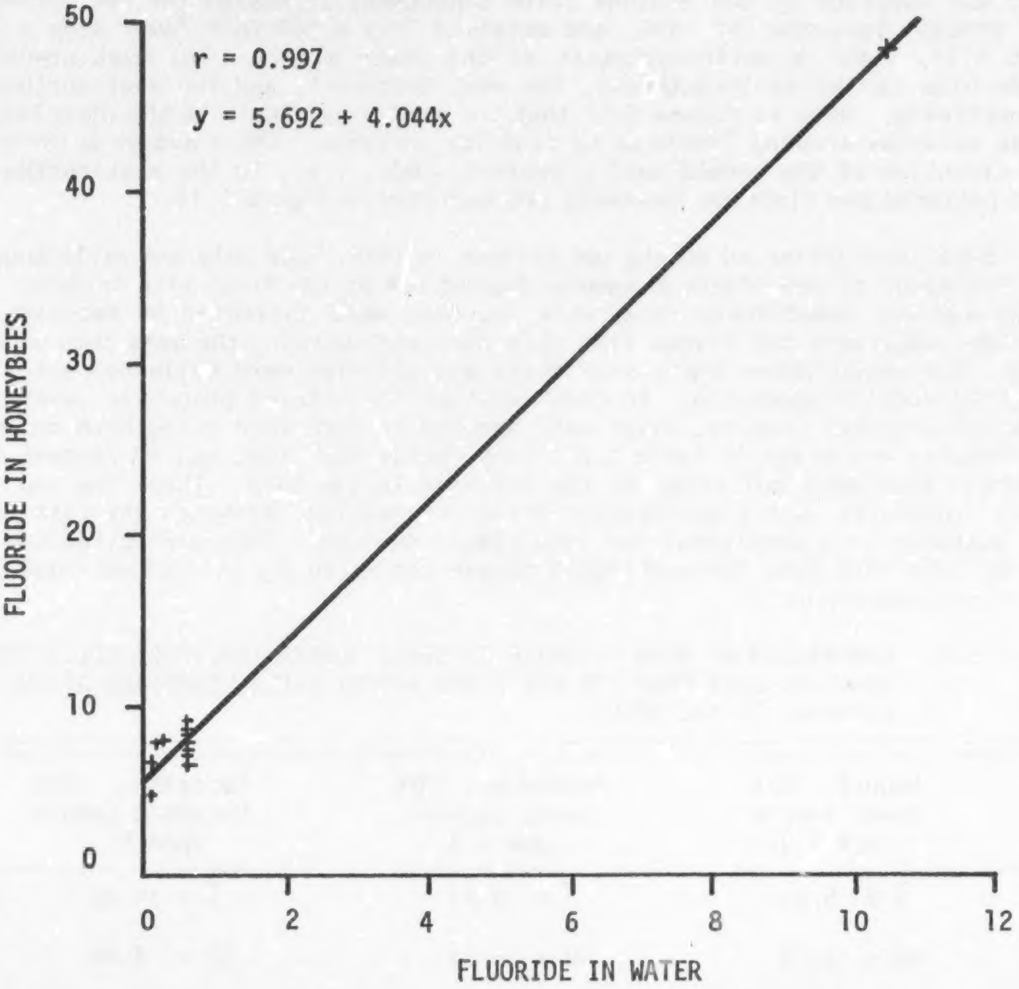


Figure 5.9. Fluoride in adult worker honeybees vs. fluoride in water.

Tables I.1, I.2, I.3, and I.4 in Appendix I present the analysis of variance for fluoride in bees collected in August of 1975, just before the first power generating unit went on-line, and for bees collected in August of 1976 (both Colstrip units have been operating at approximately one-third capacity). The data demonstrates significant increases of fluoride in bees at sites S-5, SE-1, NE-2, NE-3, and NE-4, southeast and northeast of Colstrip, while fluoride in bees at other sites remained about constant or decreased (Figure 5.10). Wind data was supplied by the Montana State Department of Health for the period of May through September of 1976, and obtained from a 300-foot tower atop a 200-foot hill, 1,400 m north-northeast of the power plants. The most prevalent winds blow to the east-southeast, the west-northwest, and the east-northeast, respectively. Note in Figure 5.11 that the wind blows least in the direction of those apiaries showing little or no fluoride increase. There are no apiaries in the direction of the second most prevalent winds, i.e., to the west-northwest. Wind patterns and fluoride increases are depicted in Figure 5.11.

Bees were collected during two periods in 1976, late July and early August. The fan motor of the electric sampler burned out at the first site in July. The motor was not immediately repairable, so bees were collected by removing the outside, uppermost two frames from each hive and sweeping the bees into plastic bags. Two weeks later these same hives and apiaries were collected using the repaired suction apparatus. To check whether the altered procedure (sweeping) affected analyses results, hives were sampled at each yard using both methods. The results are given in Table 5.8. Bees inside the hives had 50 percent less fluoride than bees collected at the entrance to the hive. Thus, the two sampling procedures gave significantly different results, although the results of the sweeping were consistent for both sample periods. This prohibited the use of the July 1976 data for meaningful comparisons with any collection taken with the vacuum apparatus.

TABLE 5.8. COMPARISON OF MEAN FLUORIDE IN ADULT WORKER HONEYBEES COLLECTED BY SWEEPING BEES FROM THE WAX COMBS AND BY VACUUM SAMPLING AT THE ENTRANCE TO THE HIVES.

| August, 1976 Sweep Sample (ppm $F^-$) | September, 1976 Sweep Sample (ppm $F^-$) | September, 1976 Vacuumed Sample (ppm $F^-$) |
|---|---|---|
| $\bar{x}$ = 5.83 | $\bar{x}$ = 5.87 | $\bar{x}$ = 12.05 |
| SD = 3.25 | SD = 3.88 | SD = 4.48 |
| SE = 0.42 | SE = 1.12 | SE = 0.67 |
| N = 61 | N = 12 | N = 45 |

Honeybee colonies have a division of labor, depending for the most part on the maturation of the worker bees. The eldest bees in a colony are the foraging bees, the bees most likely to be actively flying in and out of the entrance of the hive. Bees building comb, receiving nectar from foragers, and removing

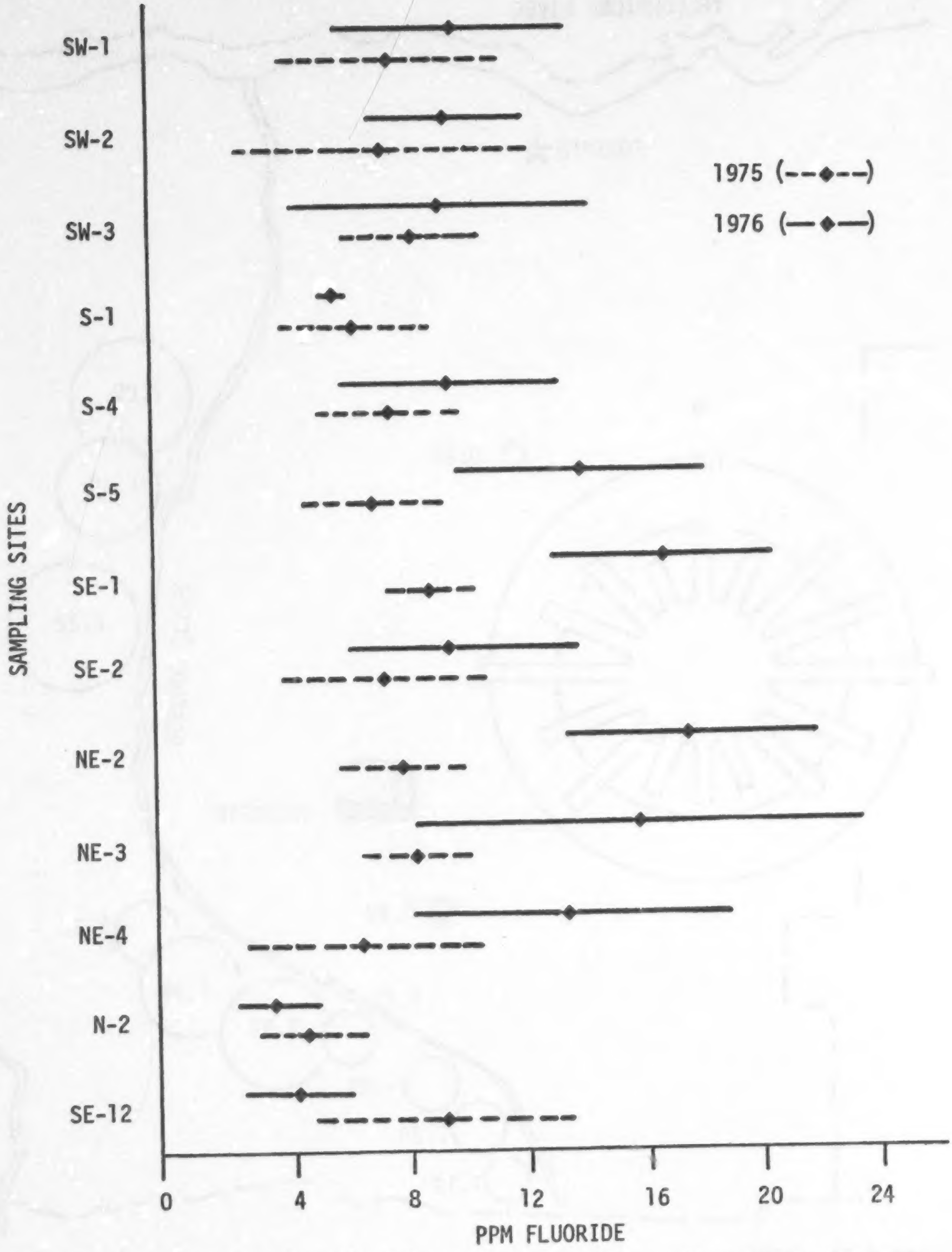


Figure 5.10. Mean fluoride in adult worker honeybees, each value is the mean of four independent samples with 95% confidence interval.

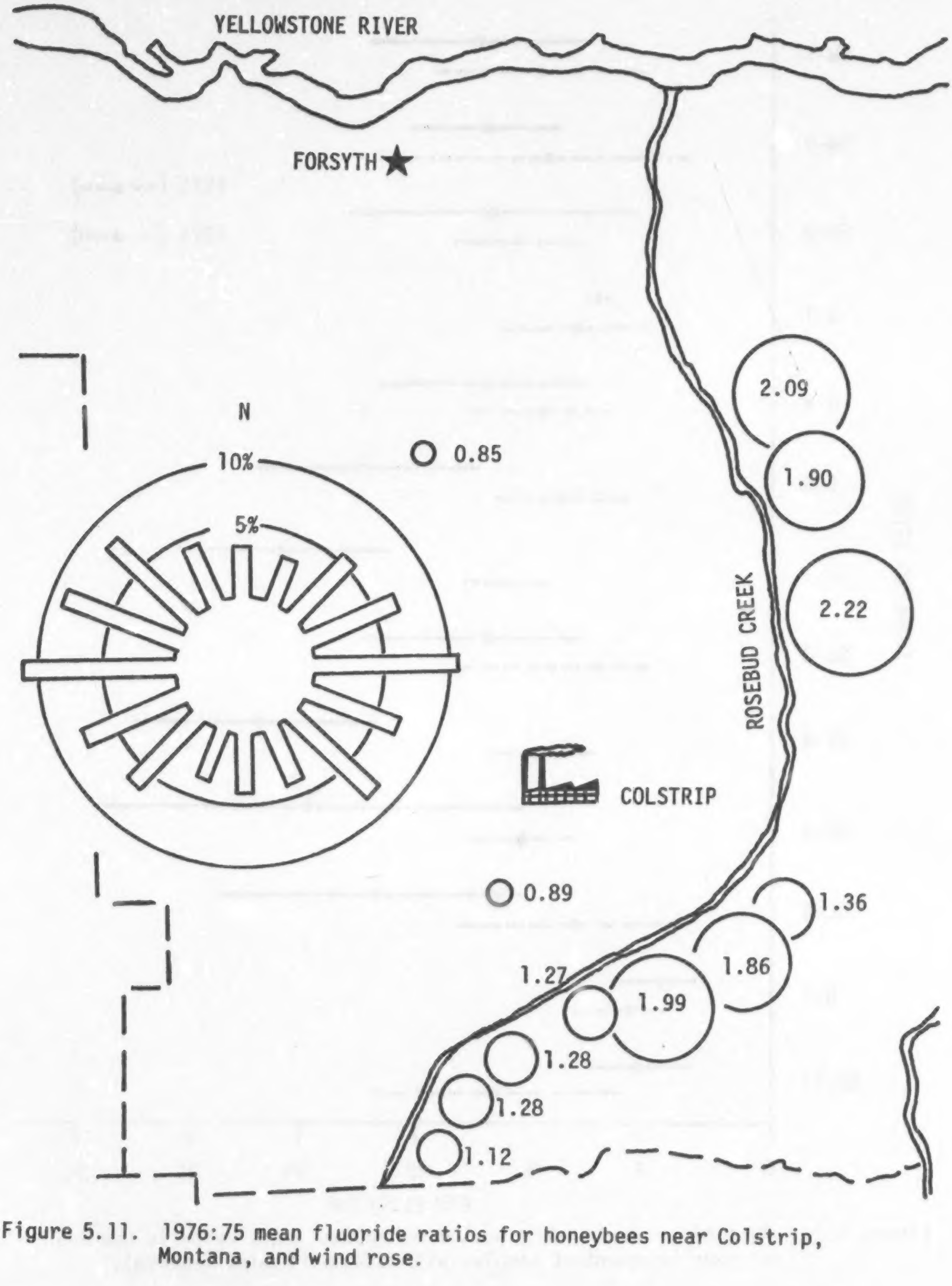

YELLOWSTONE RIVER
FORSYTH
N
10%
5%
0.85
2.09
1.90
2.22
ROSEBUD CREEK
COLSTRIP
0.89
1.36
1.86
1.27
1.99
1.28
1.28
1.12

Figure 5.11. 1976:75 mean fluoride ratios for honeybees near Colstrip, Montana, and wind rose.

debris, cell caps, and materials from the bottom of cells from the hive, tend to be of intermediate age. Differences in the average activities of individuals of a given age may occur among colonies or within the same colony at different times (Michener, 1974).

In 1975 and 1976, honey samples were collected from the same hives from which pollen and bees were obtained. Honey analyses are in process, but we are not satisfied with the efficacy of the analyses procedures. Honey is extremely hygroscopic and as such requires special handling to remove the water. This has been accomplished via acid digestion and subsequent drying. Hillmann (1972) utilized a relatively complex procedure to analyze honey with the Leco furnace, which required continual readjustments of oxygen flow and of rheostat settings, and which was less than satisfactory because of occasional explosions which sent glass flying around the laboratory (personal communication, R. Hillmann). Currently we have been searching the literature and experimenting in an effort to improve the honey analyses.

Sulfur analyses for the three years yielded values of 4,392 ± 286 (SE = 82.3), 4,000 ± 436 (SE = 251), 4,881 ± 497 (SE = 143.64) ppm, respectively. These differences were not significant ($p > .05$). Hillmann (1972) failed to detect significant differences between bees fumigated for periods of nine and 14 weeks with 1, 3, and 5 ppm $SO_2$ and those from the controls. His explanation that this was due to the comparatively higher sulfur content of the protein in bee tissues, which masked any additional amounts attributable to the fumigation, may be correct. Honey, which is primarily carbohydrate in composition, may provide a means to monitor sulfur accumulation in bee systems without the complication incurred when utilizing bee tissue. This is one of the main reasons for analyzing honey, especially since a study by Tong et al. (1975) indicates that honey does accrue contaminants such as sulfur from the environment. Another approach is to analyze bee tissue for specific sulfur compounds such as sulfate and sulfite. Gunnison (1970) was able to detect significant increases (twofold) of sulfur in the hemolymph of bees after fumigation with 8.2 ppm $SO_2$ for three weeks. However, Gunnison eventually decided to use rabbits for this work because of insufficient quantities of hemolymph, and his procedures were extremely difficult and time consuming.

A third possible approach to investigating the effect of $SO_2$ on honeybees is to analyze for acetylcholinesterase (Ache) activity, which reportedly is inhibited or decreased by sulfur dioxide. Dr. R. J. Barker, USDA Bee Research Laboratory, Tucson, Arizona, conducted Ache enzyme analyses on heads or brains from bees (both foraging and dying bees) from the ZAPS and from one of the southeastern Montana control sites (Table 5.9). He found that the enzyme activity in bees from the ZAPS and the control was as high or higher than the enzyme activity of control bees from the Tucson area, and considerably higher than the enzyme activity rates of bees poisoned by organophosphate pesticides from his own research project. The failure to detect a change in the Ache activity of bees at the ZAPS may be due to the fact that many of the bees in each colony were not exposed to the sulfur dioxide gas because the hives were placed 75 m north of the plots to avoid conflicts with the investigators working on the plots. In addition, determinations of Ache activity levels in the thoracic ganglion may be a more reliable and sensitive indicator than Ache activity levels in the brain (personal communication, W. Stephen, Oregon State University). We do not intend

TABLE 5.9. COLORIMETRIC DETERMINATION[1] OF ACETYLCHOLINESTERASE ACTIVITY IN HEADS OF HONEYBEES FROM ZAPS AND COLSTRIP PLOTS.

| Sample[2] | Number of Heads | Weight of Heads (g) | Buffer (ml) | Rate | Rate/ml | Rate/head[3] |
|---|---|---|---|---|---|---|
| A 900 | 5 | 0.0428 | 6.4 | 5.6 | 28.0 | 35.84 |
| A 901 | 5 | 0.0408 | 6.1 | 5.1 | 25.5 | 31.11 |
| A 902 | 5 | 0.0477 | 7.2 | 5.2 | 26.0 | 37.44 |
| A 903 | 5 | 0.0463 | 7.0 | 5.3 | 26.5 | 37.10 |
| A 904 | 5 | 0.0475 | 7.1 | 5.6 | 28.0 | 39.76 |
| A 905 | 5 | 0.0396 | 5.9 | 6.6 | 33.0 | 38.94 |
| A 906 | 5 | 0.0389 | 5.8 | 5.8 | 29.0 | 33.64 |
| A 907 | 5 | 0.0282 | 4.2 | 7.1 | 35.5 | 29.82 |
| A | 5 | 0.071 | 2.6 | 3.7 | 18.5 | 9.62 |
| B | 5 | 0.0323 | 4.8 | 1.5 | 7.5 | 7.2 |

[1] R. J. Barker, USDA-ARS, Tucson, Arizona.

[2] Samples A 900 - A 907 from Eastern Montana, Samples A and B from Phoenix. Phoenix bees were poisoned by pesticides.

[3] All rates for bees from ZAPS are normal.

to pursue the feasibility of Ache as an indicator of sulfur dioxide effects and/or toxicity until it is ascertained (through methods such as the use of sulfation plates or via sulfur in honey) that the bees are being exposed to the sulfur dioxide either near Colstrip or at the ZAPS (see ZAPS report).

Bees were collected for pesticide analysis primarily from the area along the Rosebud Creek drainage near Colstrip, although bees were also obtained during 1975 from apiaries near the communities of Rosebud, Broadus, Ashland, Fort Howes, and Biddle. The localities sampled in 1975 were distributed over a broader area than those sampled in 1974 to provide a means of detecting and monitoring pesticides throughout the study region. Bees from the following sites were analyzed for pesticides:

| 1975 | 1974 |
|---|---|
| BNE-2 | BNE-3 |
| BNE-3 | BNE-4 |
| BNE-4 | BNE-5 |
| BNE-5 | BSE-1 |
| BNE-10 | BSE-2 |
| BSE-1 | BS-1 |
| BSE-2 | BS-4 |
| BSE-9 | BS-5 |
| BSE-10 | BSW-2 |
| BSE-12 | BE-1 |
| BS-4 | BN-1 |
| BS-5 | |
| BS-9 | |
| BSW-2 | |

All of the 1975 samples and seven of the 1974 samples contained traces (0.01 to 0.03 ppm) of the DDT/DDD/DDE chlorinated hydrocarbon complex. Thomas reports that this is not an unusual finding in environmental samples. There was no gross contamination in any of the samples. No residues of carbamate or organophosphate pesticides were found. Because pesticide analyses require large amounts of bee tissue, it was decided to discontinue routine pesticide sampling and restrict samples utilized for pesticides to those apiaries at which symptoms of bee poisoning were observed.

The results of the analyses of honeybees and pollen for the last three years have confirmed the original hypothesis that honeybees are useful in the detection and collection of toxic materials (fluoride) from the environment. Significant (up to two times) increases were detected in the mean fluoride in bees at several apiaries located 11 to 15 km southeast and northeast of Colstrip over the average fluoride levels for the two years prior to the operation of the Colstrip power plants. Fluoride levels in water apparently play a role in the baseline levels of fluoride; fluoride in pollen may play a less important role, although Maurizio (1956) thought that bees poisoned by industrial effluents in Switzerland were obtaining fluoride via the pollen and not via water. Toshkov et al. (1974) found that bees transported poisonous substances (e.g., copper, zinc, and phosphorous) from the environment into the hives mainly by means of pollen and, to a much lesser degree, by means of nectar. They did not report analyses of water supplies. It is probable that the routes by which fluoride enters a bee colony may be varied and rely on the forms (e.g., gas, particulate, liquid) of the contaminant. Debackere's review (1972) of air pollution and apiculture makes the following observations: (1) The gaseous forms of fluoride, such as hydrogen fluoride and silicates of fluoride, are more dangerous to bees than the powdery compounds; (2) there is considerable disagreement over the toxicity of industrial fluorides to bees; (3) the diet of bees can increase manifestly their tolerance for fluorine, and (4) fluorine compounds cannot penetrate the thoracic chitin and are toxic primarily by action through the gut; for this reason, solubilized forms are said to be more toxic. He describes fluorine poisoning as a long-term mortality which can lead to the complete loss of the colony, but this loss occurs gradually, especially during the summer. The action of fluorine is that of a stomach poison; presumably fluorine can be fixed by lime particles in the epithelial cells of the digestive tract, and this exerts a protective function. The latter action would explain the increased tolerance conveyed by albumen feeding and would also explain the increased susceptibility of bees infected with Nosema disease, which affects the epithelial cells of the intestine. The long-term course of fluorine poisoning of bees could account for the lack of vigor, weak colonies, and poor production by the bees at Rosebud. The levels of fluoride in these bees approached that considered to be definitely toxic ($LD_{50}$) if the fluoride is in a soluble form, which is the form of fluoride that is measured in the water tested with the Orion specific ion electrode.

The mean fluoride level in bees from Billings, an area known to be subjected to fluoride pollution, was 30 ppm, which is approximately the level that has been reported indicative of a permanent or intermittent source of fluoride contamination (Guilhon, 1962). This is about five times higher than the levels found in Colstrip prior to the operation of the Colstrip units, and is about three times as high as the average fluoride in bees near Colstrip in 1976.

Sulfur accumulation in bees does not appear to be particularly useful in discerning accumulative effects, probably because of a masking effect by the high sulfur content of the protein in bee tissues. The results of the fluoride studies have encouraged us to examine the accumulation of other major and trace elements (such as arsenic, cadmium, and lead) which are known to be emitted during the combustion of coal.

Honeybees have been advocated previously (Toshkov et al., 1974) as bioindicators of impurities in the environment. Toshkov reports that honeybees may forage as far as 6 to 8 km from the hive or over an area of 10,000 acres. Our data shows significant increases in fluoride in bees from those apiaries located along major wind pathways from Colstrip. If this fluoride is coming from the Colstrip units, then fluorides are being carried at least 7 to 10 km from the power generators, even considering the extent of the bees' flight range. Based on the results of our current studies, honeybees appear to be excellent indicators of fluorides in the environment. In fact, they are sensitive to changes of a few parts per million and may be accumulators and magnifiers of fluoride, at least as evidenced by the magnitude of increase in bees while the levels of fluoride in water and pollen remained about constant.

If the available resources of bees are not strained in an apiary by conducting analyses which require large numbers of specimens, such as pesticides analysis, one should be able to take a greater number of individual samples and improve the usefulness of the data both in terms of improving the sample size and in terms of obtaining a better estimate of variability. One of the possible effects of increased fluoride in the system may be an increase in the variation within an apiary, since bees from each hive tend to forage in a specific area. Thus, the individuals from any one hive could be exposed to a different level of fluoride in the environment. At baseline levels, this variability may be less as each colony is exposed to a relatively constant or stable level of the contaminant. It is our intention to increase the number of individual samples and to reinstate collections at different times of the growing season.

## SUMMARY AND CONCLUSIONS

Insect and plant studies have been carried out since August 1975 to establish baseline data on populations of selected species of indigenous insects and plants. The major hypothesis was that one could develop methods to predict the bioenvironmental impacts of coal-fired power plants before damage occurred. These methods were based on the use of indicator species of insects and plants as early warning systems and as continuous monitors of atmospheric pollutants. This approach was unique because it allowed baseline studies in an area that was one of the cleanest, most unpolluted areas in the contiguous United States before the Colstrip power plants began operations (EPA, 1976; DHES, 1976; DNRC, 1975; WES, 1973). These studies and those of numerous other investigators, both under this EPA-sponsored project or other federal, state, and private sponsorship, have provided extensive baseline data of characteristics of this clean or pristine area. Almost all published studies concerning air pollution impacts on terrestrial ecosystems have either dealt with post-impact studies, which lacked pre-impact baseline information, or with controlled fumigations in laboratory chambers.

We believed (and believe) that biological indicators would show the actual responses through time of organisms or populations to individual and synergistic effects of combined pollutants or stresses. However, the bioindicators had to be sensitive to perturbations, easy to measure and monitor, and reliable predictors of bionomic and economic impacts. To test our hypothesis and to meet these criteria, we proposed and completed these objectives: (1) Selection and establishment of permanent study sites distributed to maximize gradient exposures to emissions from the Colstrip complex; (2) compilation of inventories of insect populations, infestations, and damage to indigenous plant species at the study sites to select specific groups for use as bioindicators; (3) analyses and selection of insect populations with a diversified but understandable interrelationship with indigenous plant species in order to ascertain effects of air pollutants on symbiotic associations; (4) selection and testing of disease- and injury-causing insect species for more intensive study; (5) selection and testing of beneficial insects for more intensive study, and (6) chemical analyses of selected plants and insects to establish baseline levels and to discern any accumulation of materials (mainly fluorides and sulfurs) released by the combustion of coal.

For the entomological investigations, 17 sites in ponderosa pine-skunkbush ecosystems were established on exposed ridgetops facing Colstrip, and 25 apiaries on the river and creek drainages along the interface between the grassland and the cropland ecosystems. The investigators from the Colorado National Resources Ecology Laboratory inventoried and quantified population characteristics of arthropods in the grassland areas. Therefore, we decided to compile information concerning the major or dominant insect populations on ponderosa pines, a species which research has shown (Gordon et al., 1976; Gordon et al., 1977) to be one of the most sensitive plant species to the phytotoxic gases released by coal-burning facilities. This pine is dominant throughout much of southeastern Montana and is the sole commercial timber resource. In addition, two points were of critical importance: (1) Many reports indicated that conifers weakened or injured by pollutants are more likely to incur attack from insects that prey and reproduce on weakened trees (Haegle, 1973), and (2) phytophagous insects have long-term interactions with fundamental ecosystem processes such as primary production and nutrient cycling (Mattson and Addy, 1975). We quantified pathologies of trees and foliage and established data banks concerning baseline levels of sulfur, fluorides, and trace elements. The insect surveys identified prevalent species of both pest and beneficial insects associated with the pine, evaluated the status of insect pests and their immediate potential for outbreak or epidemic, determined which insect species most likely were responsible for insect-induced pathologies, and provided histopathological demonstrations of insect damage to pine foliage. The latter were of particular value when juxtaposed with the histopathological micro-slide series of fungal, pollutant, and abiotic (e.g., frost, drought) damage syndromes. Histopathological descriptions allowed us to "fingerprint" damage from the initial damage through each stage as it first becomes visible and then fully manifest. We already have a "fingerprint" file for many types of injury from phytotoxic gases and from fungi. We quantified foliage damage by insects and other sources, rather than attempting to quantify the absolute size of pest insect populations. Ultimately, it was the amount of injury that was the most meaningful measure of the effect of an insect population on its host. Based on pre-operational studies, the southeastern Montana area was considered to be exceptionally free of serious insect problems, as evidenced both by insect and disease reports and by our own observations.

One insect, identified as a Matsucoccus scale, is of the highest interest because it resides at the base of the needles under the fascicular sheath, is often seen near areas of basal necrosis or basal burn to the needles, as is typical of acid rain damage, and is reported to increase in numbers in areas of pollution stress (Siewniak, 1971). The life system of this insect was concentrated on, including its interactions with ponderosa pine, and the pathological symptoms of its feeding and attack. This is an ongoing objective; its relationships with ponderosa pines are still not fully understood. The results of this work should make a significant contribution not only towards understanding the role air pollution plays in the population dynamics of this organism but also to an understanding of the basic biology of this insect, which is seldom studied by researchers in the United States and Canada, although some species have caused extensive damage in the United States (Keen, 1952).

The post-operational investigations did not demonstrate any significant increase in insect pests or insect damage at any of the sites, but our chemical analyses and foliage pathology determinations demonstrated significant increases in sulfur and fluoride in plant foliage at two sites and significant increases in needle pathologies of 14- to 26-month-old needles collected from one of these sites (see section by Gordon et al., this report).

Honeybees were utilized near Colstrip as accumulators and indicators of the extent and magnitude of the phytotoxic and zootoxic effluents from the Colstrip power units. The 1974, 1975, and 1976 honeybee collections demonstrated significant differences among sites in the fluoride content of bees. However, the pre-operational levels of fluoride in bees prior to the operation of the Colstrip units averaged 8.5 ppm for 22 sites in 1975 and 7 4 ppm for 11 sites in 1974. One site was shown to have a source of fluoride contamination (10 to 12 ppm) in the bees' water supply, and the bees at that site averaged 96 ppm for all years. Honeybees collected at apiaries approximately 15 to 20 km northeast and southeast of Colstrip in September 1976 (12 months after Colstrip Unit 1 went on-line), demonstrated a significant ($p \leq .001$) fluoride increase over the levels found at these sites during the previous two years. Increases as high as twofold occurred at these sites, which are in the path of frequent winds from Colstrip, while slight decreases in fluoride were demonstrated at sites north and south of Colstrip in the path of the least frequent winds. Both 1975 and 1976 pollen samples averaged 1.9 ppm for all sites. Fluoride in water at the sites, which hypothetically could be responsible for significant increases of fluoride in bees, was practically constant at 0.6 ppm. Since honeybees may forage up to 6 to 8 km (Toshkov et al., 1974) from their hives, this would mean that if the bees are accumulating fluoride emitted by the Colstrip units, these effluents are being carried at least 7 to 10 km southeast and northeast of Colstrip.

The studies indicated that honeybees may be extremely sensitive and useful indicators of fluorides in the environment, and this prompted us to design a study for the 1977 field season investigating the presence of other major and trace elements. Honeybees do not appear to be particularly useful for the detection of sulfurs in the environment because the natural levels of sulfur in bee tissues are so high that they apparently mask any measurable increases. The results of analyses of honeybees for the presence of pesticide residues in 1974 and 1975 revealed only low levels (0.01 to 0.03 ppm) of chlorinated hydrocarbons in any of the samples, a result that is not unusual in these types of samples.

The results of the baseline studies of the accumulation of phytotoxic substances of anthropogenic origin in vegetation, honeybees, and pollen; the field surveys of pest insect populations and infestations on ponderosa pines and associated measurements of foliage pathologies; and the field studies of insect-plant life systems (namely M. secretus on ponderosa pine) reaffirm the belief that one should be able to follow changes in the baseline characteristics of the pine and grassland ecosystems of southeastern Montana as they are exposed to emissions from the Colstrip power plants.

Furthermore, it is believed that these entomological investigations contribute to both basic and applied fields of air pollution research about changes in systems of insects and insect-plant interactions and about the pathways of air pollutants through an insect system, honeybees in this case. The information on the responses of pollinators to effluents from coal-fired generating plants should be invaluable to other EPA studies on plant diversity, plant productivity, and plant community changes, while the work on the ponderosa pine systems adds this prominent ecosystem to the grassland ecosystem studies of the overall project.

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## APPENDIX B

## HONEYBEE COLLECTION SITES IN THE FORT UNION BASIN

| Site No. | Location | Site No. | Location |
|---|---|---|---|
| BN-1 | T3N, R41E, SEC 28 | BSE-6 | T2S, R46E, SEC 34 |
| BN-2 | T4N, R40E, SEC 26 | BSE-7* | T2S, R47E, SEC 19 |
| BS-1 | T1N, R41E, SEC 24 | BSE-9 | T4S, R51E, SEC 25 |
| BS-4 | T1S, R42E, SEC 8 | BSE-9a* | T4S, R51E, SEC 30 |
| BS-5 | T1S, R42E, SEC 3 | BSE-10 | T8S, R51E, SEC 20 |
| BSE-8 | T3S, R45E, SEC 18 | BE-1 | T2N, R43E, SEC 16 |
| BSE-11 | T7S, R47E, SEC 9 | BNE-2 | T3N, R43E, SEC 33 |
| BSE-12 | T7S, R46E, SEC 6 | BNE-2a | T3N, R43E, SEC 21 |
| BSW-1 | T1S, R41E, SEC 13 | BNE-3 | T3N, R43E, SEC 8 |
| BSW-2 | T1S, R41E, SEC 22 | BNE-4 | T4N, R43E, SEC 32 |
| BSW-3 | T2S, R41E, SEC 4 | BNE-5 | T4N, R42E, SEC 13 |
| BSE-1 | T1N, R42E, SEC 26 | BNE-6 | T5N, R42E, SEC 5 |
| BSE-2 | T1N, R43E, SEC 19 | BNE-10 | T6N, R42E, SEC 16 |

*Wild honeybee colonies.

# APPENDIX C

## DIRECTIONS FOR PONDEROSA PINE-INSECT STUDY

### I. Field Procedures

A. Record the plot location, date of collection, and number of the tree being sampled in the field diary.

B. Prepare a collection card with the same information as in the previous step, and place the card in a new plastic collection bag.

C. Classify the tree based on condition (modification of the procedure of Carlson _et al_., 1974), and record in diary.

Tree Class

| | |
|---|---|
| 0 | No apparent insect feeding or pollution damage. Needle retention appears normal. |
| 1 | Some damage evident: light insect activity, foliar burn restricted to older needles, or crowns becoming thin. Needle retention below normal. |
| 2 | Damage by insect feeding _quite_ evident, foliar burn conspicuous, or thin crown. Needle retention below normal or poor. |
| 3 | Heavy insect damage evident. Excessive insect feeding and/or foliar burn. Crowns thin and needle retention poor. |
| 4 | Tree is dead or dying. |

D. Estimate height of tree and measure diameter at breast height; record in field diary.

E. Inspect the base, trunk, crown, branches, and twigs of the tree for insects and injury (includes damage by storms, porcupines, or other agents). Record observations and place any insects into a labeled vial filled with 80% ethanol to which has been added a drop of glycerol.

APPENDIX C (continued)

F. Beat both the upper and lower foliage of the tree with a sweep net (50 sweeps/level). Preserve any insects obtained in alcohol (paper Lepidoptera); list any insects in field diary. If insect has not been identified, give a brief written description and indicate order or family.

G. Examine 100 mature cones (fewer if necessary) on the tree and record the percent of the cones dwarfed or damaged by insect feeding; identify the damaging insect(s).

H. Remove four branches (five internodes/branch) from top one half and four branches from the lower one half of the foliage from side of tree facing Colstrip. Place the branches from each of the two levels in separate plastic bags. Keep the samples out of the sun, covered by a tarp or in a canvas bag. Process or refrigerate as soon as possible.

I. If any dead or dying trees are visible from the marked sample tree, inspect the trees and record the probable cause(s) of death.

II. Laboratory Procedures for each Sample

A. Cut branches from a given tree into internode segments to separate the foliage by age.

B. From the four most recent ages, select four internodes for determinations of percent needle retention.

C. Remove all fascicles and maintain by age.

D. Randomly select 100 fascicles of each age, remove the sheaths, and save 100 needles.

E. For each of these 100 needles, inspect for each of the various types of injury; including basal necrosis, basal scale (unidentified), pine needle scale (Phenacaspis pinifoliae, defoliators, tip necrosis, chlorotic mottle, and weevil) and record the percentage of needles affected by each agent.

F. Save representative specimens of different pathology types; kill and fix in FAA (formalin-aceto-alcohol).

G. Save any insects in 80% ethanol, label, record any comments.

H. Complete measurements of cross-sectional area, needle length, and moisture percentage.

I. Prepare all stripped needles for chemical analyses.

## APPENDIX D

## CHECKLIST OF PREVALENT INSECTS ASSOCIATED WITH PONDEROSA PINES IN SOUTHEASTERN MONTANA

| Order | Family | Species |
|---|---|---|
| Neuroptera | Mantispidae | Climaciella brunnea (Say) |
| " " | Chrysopidae | Chrysopa sp. |
| Homoptera | Aphidae | Cinara ponderosae (Wms.) |
| " " | Coccidae | Matsucoccus secretus Morrison |
| " " | " " | Phenacaspis pinifoliae (Fitch) |
| Hemiptera | Coreidae | Leptoglossus sp. |
| Coleoptera | Buprestidae | Buprestis spp. |
| " " | Cerambycidae | Arthopalus foreicollis (Lec.) |
| " " | " " | Batyle Thomson sp. |
| " " | " " | Monochamus maculosus Hald. |
| " " | " " | Neoclytus muricatulus (Kby.) |
| " " | " " | Prionus imbricornis (L.) |
| " " | Coccinellidae | Anatis quindecimpunctata (Oliv.) |
| " " | " " | Cleis picta (Rand.) |
| " " | " " | Hippodamia convergens Guerin |
| " " | " " | Neomysia Casey sp. |
| " " | Curculionidae | Magdalis Germar spp. |
| " " | " " | Pissodes probably fasciatus Hopk. |

APPENDIX D (continued)

| Order | Family | Species |
|---|---|---|
| Coleoptera | Curculionidae | *Scythropus elegans* (Couper) |
| " " | " " | *Scythropus* probably *albidus* Fall |
| " " | Scarabaeidae | *Cotalpa lanigera* Wick. |
| " " | " " | *Polyphylla decimlineata* (Say) |
| " " | Scolytidae | *Conophthorus* Hopk. probably *ponderosae* |
| " " | " " | *Dendroctonus ponderosae* Hopk. |
| " " | " " | " " *valens* LeConte |
| " " | " " | *Dryocoetes* probably *confuses* Sw. |
| " " | " " | *Hylurgops subcostulatus* (Mann.) |
| " " | " " | *Ips calligraphus* (Germar) |
| " " | " " | *Ips pini* (Say) |
| " " | " " | *Pityophthorus* sp. |
| " " | " " | *Scolytus* sp. |
| Diptera | Cecidomyiidae | Unidentified species |
| " " | Tachinidae | *Euphorocera* sp. near *edwardsii* (Williston) |
| Lepidoptera | Arctiidae | *Dasychira* near *grisefacta* Dyar |
| " " | " " | *Hypantria cunea* (Drury) |
| " " | Gelechiidae | *Coleotechnites* sp. |
| " " | Geometridae | *Phaeoura mexicanaria* (Grote) |
| " " | Lasiocampidae | *Malacosoma disstria* Hbn. |
| " " | Olethreutidae | *Laspeyresia* sp. |
| " " | " " | *Petrova* sp. |
| " " | " " | *Rhyacionia* (Busck) sp. |
| " " | Pyralidae | *Dioryctria abietella* (D.&S.) |

APPENDIX D (continued)

| Order | Family | Species |
|---|---|---|
| Lepidoptera | Pyralidae | *Dioryctria* spp. |
| " " | Pieridae | *Neophasia menapia* Felder |
| " " | Yponomeutidae | *Zellaria* probably *haimbachi* Busck |
| Hymenoptera | Diprionidae | *Diprion* sp. |
| " " | " " | *Neodiprion fulviceps* complex |
| " " | Ichneumonidae | *Ichneumon pulcherior* (Heinrich) |
| " " | Pteromalidae | *Coelopisthia suborbicularis* (Provancher) |
| " " | Vespidae | *Vespula* spp. |

# APPENDIX E

## PRE-OPERATIONAL ON SITE MEASUREMENTS

| F Number | Site | Approx. Height (ft) | Diameter Breast Height (in) | % Dwarfed Cones (50-100 cones/tree) | Tree Class (0-4) 1974 | Tree Class (0-4) 1975 | Porcupine Attack (trunk and/ or limbs) |
|---|---|---|---|---|---|---|---|
| F-1300 | N #4 | 22 | 11.9 | 2 | 0 | 0 | NE* |
| F-1300.5 | " | 24 | 10.8 | 7 | - | 0 | P† |
| F-1305 | " | 18 | 14.6 | 3 | 0 | 0 | NE |
| F-1305.5 | " | 27 | 12.6 | 0 | - | 0 | NE |
| F-1310 | " | 24 | 18.0 | 32 | 0 | 0 | NE |
| F-1310.5 | " | 30 | 17.7 | 0 | - | 0 | NE |
| F-1315 | " | 27 | 15.4 | 6 | 0 | 0 | NE |
| F-1315.5 | " | 23 | 15.3 | 2 | - | 0 | NE |
| F-1320 | " | 25 | 12.1 | 3 | 0 | 0 | R‡ |
| F-1320.5 | " | 24 | 11.1 | 0 | - | 0 | P |
| F-1850 | NE #1 | 42 | 21.3 | 20 | 0 | 0 | NE |
| F-1850.5 | " | 42 | 15.9 | 18 | - | 0 | NE |
| F-1855 | " | 43 | 15.8 | 20 | 0 | 0 | NE |
| F-1855.5 | " | 41 | 18.1 | 34 | - | 0 | NE |
| F-1860 | " | 24 | 11.1 | 42 | 0 | 0 | NE |

*Not evident

†Previous

‡Recent (within last 12 months)

APPENDIX E (continued)

| F Number | Site | Approx. Height (ft) | Diameter Breast Height (in) | % Dwarfed Cones (50-100 cones/tree) | Tree Class (0-4) 1974 | Tree Class (0-4) 1975 | Porcupine Attack (trunk and/ or limbs) |
|---|---|---|---|---|---|---|---|
| F-1860.5 | NE #1 | 30 | 14.3 | 37 | - | 0 | NE |
| F-1865 | " | 45 | 16.7 | 10 | 0 | 0 | NE |
| F-1865.5 | " | 45 | 16.4 | 14 | - | 0 | NE |
| F-1870 | " | 27 | 11.0 | 26 | 0 | 0 | P |
| F-1870.5 | " | 30 | 11.5 | 26 | - | 0 | NE |
| F-1575 | NE #3 | 36 | 16.6 | 3 | 0 | 0 | R |
| F-1575.5 | " | 29 | 14.8 | 1 | - | 0 | R |
| F-1580 | " | 38 | 11.9 | 3 | 0 | 0 | P |
| F-1580.5 | " | 32 | 15.4 | 5 | - | 0 | R |
| F-1585 | " | 23 | 9.5 | 0 | 0 | 1 | P |
| F-1585.5 | " | 20 | 9.2 | 0 | - | 1 | P |
| F-1590 | " | 19 | 10.5 | 0 | 0 | 0 | R |
| F-1590.5 | " | 30 | 11.9 | 0 | - | 1 | NE |
| F-1595 | " | 23 | 11.6 | 0 | 0 | 1 | NE |
| F-1595.5 | " | 20 | 9.4 | 0 | - | 1 | P |
| F-1775 | NW #3 | 45 | 21.6 | 76 | 0 | 0 | R |
| F-1775.5 | " | 42 | 20.7 | - | - | 0 | P |
| F-1780 | " | 39 | 16.6 | 45 | 0 | 0 | P |
| F-1780.5 | " | 32 | 14.3 | 60 | - | 1 | P |
| F-1785 | " | 40 | 17.0 | 68 | 1 | 0 | NE |
| F-1785.5 | " | 50 | 20.2 | - | - | 0 | NE |

APPENDIX E (continued)

| F Number | Site | Approx. Height (ft) | Diameter Breast Height (in) | % Dwarfed Cones (50-100 cones/tree) | Tree Class (0-4) 1974 | Tree Class (0-4) 1975 | Porcupine Attack (trunk and/ or limbs) |
|---|---|---|---|---|---|---|---|
| F-1790 | NW #3 | 33 | 14.0 | 60 | 0 | 0 | P |
| F-1790.5 | " | 35 | 11.1 | 62 | - | 0 | P |
| F-1795 | " | 37 | 20.1 | 73 | 0 | 0 | P |
| F-1795.5 | " | 41 | 18.8 | 37 | - | 0 | P |
| F-1350 | NW #4 | 28 | 10.2 | - | 1 | 0 | NE |
| F-1350.5 | " | 29 | 11.3 | 30 | - | 0 | P |
| F-1355 | " | 44 | 20.1 | - | 1 | 0 | NE |
| F-1355.5 | " | 26 | 13.2 | 13 | - | 0 | P |
| F-1360 | " | 32 | 15.3 | 55 | 1 | 0 | NE |
| F-1360.5 | " | 31 | 15.9 | - | - | 0 | NE |
| F-1365 | " | 34 | 14.3 | 48 | 1 | 0 | NE |
| F-1365.5 | " | 20 | 17.3 | 44 | - | 0 | NE |
| F-1370 | " | 38 | 20.1 | 60 | 0 | 0 | NE |
| F-1370.5 | " | 28 | 16.2 | 33 | - | 0 | NE |
| F-1750 | W #3 | 35 | 13.7 | 25 | 0 | 0 | NE |
| F-1750.5 | " | 32 | 12.6 | - | - | 0 | NE |
| F-1755 | " | 42 | 19.4 | - | 0 | 1 | R |
| F-1755.5 | " | 31 | 13.5 | - | - | 0 | P |
| F-1760 | " | 54 | 10.2 | 23 | 0 | 0 | P |
| F-1760.5 | " | 48 | 10.5 | 32 | - | 1 | NE |
| F-1765 | " | 26 | 10.0 | - | 0 | 0 | NE |

APPENDIX E (continued)

| F Number | Site | Approx. Height (ft) | Diameter Breast Height (in) | % Dwarfed Cones (50-100 cones/tree) | Tree Class (0-4) 1974 | Tree Class (0-4) 1975 | Porcupine Attack (trunk and/ or limbs) |
|---|---|---|---|---|---|---|---|
| F-1765.5 | W #3 | 25 | 13.2 | 64 | - | 0 | NE |
| F-1770 | " | 40 | 13.8 | 24 | 0 | 0 | P |
| F-1770.5 | " | 30 | 12.3 | - | - | - | NE |
| F-1200 | W #4 | 28 | 13.5 | 2 | 0 | 0 | NE |
| F-1200.5 | " | 26 | 9.9 | 0 | - | 0 | NE |
| F-1205 | " | 36 | 14.0 | 12 | 0 | 0 | NE |
| F-1205.5 | " | 30 | 11.0 | 3 | - | 0 | NE |
| F-1210 | " | 28 | 11.5 | 5 | 0 | 0 | NE |
| F-1210.5 | " | 24 | 11.5 | 3 | - | 0 | NE |
| F-1215 | " | 42 | 18.1 | 10 | 0 | 0 | NE |
| F-1215.5 | " | 20 | 15.9 | 3 | - | 0 | NE |
| F-1220 | " | 28 | 14.6 | 0 | 0 | 0 | P |
| F-1220.5 | " | 30 | 12.1 | 5 | - | 0 | NE |
| F-1475 | SE #1 | 29 | 12.1 | 2 | 0 | 0 | NE |
| F-1475.5 | " | 30 | 13.2 | 1 | - | 0 | NE |
| F-1480 | " | 30 | 15.9 | 22 | 0 | 0 | NE |
| F1480.5 | " | 32 | 12.3 | 21 | - | 0 | NE |
| F-1485 | " | 26 | 13.5 | 77 | 0 | 0 | NE |
| F-1485.5 | " | 28 | 14.0 | 96 | - | 0 | NE |
| F-1490 | " | 26 | 10.5 | 28 | 0 | 0 | NE |
| F-1490.5 | " | 20 | 8.6 | 29 | - | 0 | NE |

APPENDIX E (continued)

| F Number | Site | Approx. Height (ft) | Diameter Breast Height (in) | % Dwarfed Cones (50-100 cones/tree) | Tree Class (0-4) 1974 | Tree Class (0-4) 1975 | Porcupine Attack (trunk and/ or limbs) |
|---|---|---|---|---|---|---|---|
| F-1495 | SE #1 | 26 | 18.8 | 26 | 0 | 0 | NE |
| F-1495.5 | " | 26 | 12.1 | 26 | - | 0 | NE |
| F-1050 | SE #3 | 30 | 13.1 | 9 | 0 | 0 | P |
| F-1050.5 | " | 35 | 17.3 | 10 | - | 0 | NE |
| F-1055 | " | 38 | 19.3 | 4 | 0 | 0 | NE |
| F-1055.5 | " | 35 | 15.6 | 6 | - | 0 | NE |
| F-1060 | " | 26 | 14.5 | 10 | 0 | 0 | NE |
| F-1060.5 | " | 33 | 18.1 | 8 | - | 0 | NE |
| F-1065 | " | 26 | 11.5 | 4 | 0 | 0 | NE |
| F-1065.5 | " | 26 | 14.6 | 10 | - | 0 | NE |
| F-1070 | " | 36 | 16.2 | 12 | 0 | 0 | NE |
| F-1070.5 | " | 33 | 18.0 | - | - | 0 | NE |
| F-1100 | S #3 | 42 | 15.8 | - | 0 | 0 | NE |
| F-1100.5 | " | 31 | 9.9 | - | - | 0 | NE |
| F-1105 | " | 30 | 12.1 | - | 0 | 0 | NE |
| F-1105.5 | " | 34 | 13.5 | - | - | 0 | NE |
| F-1110 | " | 28 | 12.7 | - | 0 | 0 | NE |
| F-1110.5 | " | 35 | 15.1 | - | - | 0 | NE |
| F-1115 | " | 45 | 16.8 | - | 0 | 0 | NE |
| F-1115.5 | " | 29 | 11.1 | - | - | 0 | NE |
| F-1120 | " | 34 | 19.1 | - | 0 | 0 | NE |

APPENDIX E (continued)

| F Number | Site | Approx. Height (ft) | Diameter Breast Height (in) | % Dwarfed Cones (50-100 cones/tree) | Tree Class (0-4) 1974 | Tree Class (0-4) 1975 | Porcupine Attack (trunk and/ or limbs) |
|---|---|---|---|---|---|---|---|
| F-1120.5 | S #3 | 32 | 9.9 | - | - | 0 | NE |
| F-1825 | E #1 | 45 | 15.8 | 29 | 0 | 0 | NE |
| F-1825.5 | " | 51 | 14.0 | 31 | - | 0 | NE |
| F-1830 | " | 45 | 25.1 | 32 | 0 | 0 | R |
| F-1830.5 | " | 42 | 20.1 | 46 | - | 0 | NE |
| F-1835 | " | 24 | 15.0 | 41 | 0 | 0 | NE |
| F-1835.5 | " | 27 | 13.0 | 39 | - | 0 | NE |
| F-1840 | " | 32 | 20.0 | 47 | 0 | 0 | R |
| F-1840.5 | " | 25 | 11.0 | 62 | - | 0 | R |
| F-1845 | " | 38 | 20.3 | 30 | 0 | 0 | NE |
| F-1845.5 | " | 34 | 13.7 | 94 | - | 0 | NE |
| F-1275 | E #3 | 24 | 16.1 | 5 | 0 | 0 | NE |
| F-1275.5 | " | 25 | 14.2 | 0 | - | 0 | NE |
| F-1280 | " | 30 | 17.8 | 1 | 0 | 0 | NE |
| F-1280.5 | " | 24 | 18.3 | 6 | - | 0 | NE |
| F-1285 | " | 38 | 14.8 | 3 | 0 | 0 | NE |
| F-1285.5 | " | 37 | 15.6 | 1 | - | 0 | NE |
| F-1290 | " | 27 | 13.7 | 2 | 0 | 0 | NE |
| F-1290.5 | " | 37 | 15.9 | 2 | - | 0 | NE |
| F-1295 | " | 35 | 20.1 | 0 | 0 | 0 | NE |
| F-1295.5 | " | 33 | 16.6 | 1 | - | 0 | NE |

APPENDIX E (continued)

| F Number | Site | Approx. Height (ft) | Diameter Breast Height (in) | % Dwarfed Cones (50-100 cones/tree) | Tree Class (0-4) 1974 | Tree Class (0-4) 1975 | Porcupine Attack (trunk and/ or limbs) |
|---|---|---|---|---|---|---|---|
| F-1250 | E #4 | 26 | 21.0 | 0 | 0 | 0 | P |
| F-1250.5 | " | 18 | 9.5 | 3 | - | 0 | P |
| F-1255 | " | 28 | 14.3 | 3 | 0 | 0 | P |
| F-1255.5 | " | 30 | 11.6 | 6 | - | 0 | R |
| F-1260 | " | 30 | 12.3 | 3 | 0 | 0 | P |
| F-1260.5 | " | 24 | 12.1 | 2 | - | 0 | P |
| F-1265 | " | 15 | 9.4 | 0 | 0 | 0 | R |
| F-1265.5 | " | 17 | 13.2 | 1 | - | 0 | P |
| F-1270 | " | 18 | 10.7 | 0 | 0 | 0 | P |
| F-1270.5 | " | 16 | 8.9 | 0 | - | 0 | R |
| F-1225 | E #5 | 36 | 21.3 | 25 | 0 | 0 | R |
| F-1225.5 | " | 33 | 21.1 | 20 | - | 0 | R |
| F-1230 | " | 33 | 20.7 | 3 | 0 | 0 | P |
| F-1230.5 | " | 25 | 12.7 | 3 | - | 0 | R |
| F-1235 | " | 37 | 23.6 | 4 | 0 | 0 | P |
| F-1235.5 | " | 40 | 18.3 | 3 | - | 0 | P |
| F-1240 | " | 29 | 11.6 | 4 | 0 | 0 | R |
| F-1240.5 | " | 34 | 18.8 | 5 | - | 0 | NE |
| F-1245 | " | 35 | 26.1 | 5 | 0 | 0 | R |
| F-1245.5 | " | 28 | 17.2 | 6 | - | 0 | R |

## APPENDIX F

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## APPENDIX G

### TABLE G.1. PEARSON PRODUCT-MOMENT CORRELATION COEFFICIENT FOR PPM FLUORIDE IN ADULT WORKER HONEYBEES VERSUS POLLEN, 1975 AND 1976 DATA

N = 114, Chi Square = 3.841, F-Value = 3.0700000, Alpha = .0500

The Transformation Codes are 0 and 0

| | Y1 (Honeybees) | Y2 (Pollen) |
|---|---|---|
| Mean | 9.57 | 1.89 |
| Variance | 16.311 | 0.840 |
| Standard Deviation | 4.0386 | 0.9167 |
| Standard Error | 0.3782 | 0.0858 |
| Covariance | -0.395 | |

The product-moment correlation coefficient is -0.10674.

The 95.0 percent confidence limits are L1 = 0.2851 and L2 = 0.0788.

The eigenvalues are 16.32101 and 0.83035.

The equation of the principal axis is: Y1 = 83.89246 + -39.17078 Y2.

The equation of the minor axis is: Y1 = 9.52261 + 0.02553 Y2.

The 95.0 percent confidence limits to the slope of the principal axis are:
L1 = 55.20636 L2 = 14.43632

Following Pairs Show the Coordinates of Points
A to H for Plotting Confidence Ellipse.

| Number One | Y1 | Y2 |
|---|---|---|
| A | 10.5071 | 1.8974 |
| B | 8.6350 | 1.8974 |
| C | 9.5711 | 2.1099 |
| D | 9.5711 | 1.6849 |
| E | 8.6296 | 1.9214 |
| F | 10.5125 | 1.8733 |
| G | 9.5765 | 2.1097 |
| H | 9.5656 | 1.6850 |

## TABLE G.2. PEARSON PRODUCT-MOMENT CORRELATION COEFFICIENT FOR PPM FLUORIDE IN ADULT WORKER HONEYBEES VERSUS POLLEN, 1975 DATA

N = 66, Chi Square = 3.841, F-Value = 3,1400000, Alpha = .0500
The Transformation Codes are 0 and 0

| | Y1 (Honeybees) | Y2 (Pollen) |
|---|---|---|
| Mean | 8.13 | 1.90 |
| Variance | 7.346 | 0.776 |
| Standard Deviation | 2.7103 | 0.8806 |
| Standard Error | 0.3336 | 0.1084 |
| Covariance | 0.305 | |

The product-moment correlation coefficient is: 0.12801.

The 95.0 percent confidence limits are L1 = 0.1177 and L2 = 0.3590.

The eigenvalues are: 7.36038 and 0.76134.

The equation of the principal axis is: Y1 = 32.91300 + 21.55098 Y2.

The equation of the minor axis is Y1 = 8.22019 + -0.04640 Y2.

The 95.0 percent confidence limits to the slope of the principal axis are:
L1 = 7.47355 L2 = 24.81377

Following Pairs Show the Coordinates of Points
A to H For Plotting Confidence Ellipse

| Number Two | Y1 | Y2 |
|---|---|---|
| A | 8.9675 | 1.9045 |
| B | 7.2962 | 1.9045 |
| C | 8.1318 | 2.1761 |
| D | 8.1318 | 1.6330 |
| E | 8.9743 | 1.9436 |
| F | 7.2893 | 1.8655 |
| G | 8.1192 | 2.1755 |
| H | 8.1444 | 1.6336 |

TABLE G.3. PEARSON PRODUCT-MOMENT CORRELATION COEFFICIENT FOR PPM FLUORIDE IN ADULT WORKER HONEYBEES VERSUS POLLEN, 1976 DATA

N = 48, Chi Square = 3.841, F-Value = 3.1900000, Alpha = .0500
The Transformation Codes are 0 and 0

| | Y1 (Honeybees) | Y2 (Pollen) |
|---|---|---|
| Mean | 11.55 | 1.88 |
| Variance | 22.148 | 0.948 |
| Standard Deviation | 4.7061 | 0.9736 |
| Standard Error | 0.6792 | 0.1405 |
| Covariance | -1.338 | |

The product-moment correlation coefficient is: -0.29208.

The 95.0 percent confidence limits are L1 = -0.5321 and L2 = 0.0086.

The eigenvalues are: 22.23181 and 0.86378.

The equation of the principal axis is: Y1 = 41.56816 + -15.90366 Y2.

The equation of the minor axis is: Y1 = 11.43132 + 0.06288 Y2.

The 95 percent confidence limits to the slope of the principal axis are:
L1 = 210.50580 L2 = 8.23499

Following Pairs Show the Coordinates of Points
A to H For Plotting Confidence Ellipse

| Number One | Y1 | Y2 |
|---|---|---|
| A | 13.2087 | 1.8875 |
| B | 9.8913 | 1.8875 |
| C | 11.5500 | 2.2306 |
| D | 11.5500 | 1.5444 |
| E | 9.8158 | 1.9965 |
| F | 13.2842 | 1.7785 |
| G | 11.5715 | 2.2293 |
| H | 11.5285 | 1.5457 |

## APPENDIX H

## MEAN FLUORIDE IN ADULT WORKER HONEYBEES - 1975-1976.

| | | August 1975 | | | | | | August - September 1976 | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Each value represents a different hive | | | | Each value represents a combined sample from 8-12 hives | | Each value represents a different hive | | | | Each value represents a combined sample from 8-12 hives | | |
| Site | | 1 | 2 | 3 | 4 | 5 | 6 | 1 | 2 | 3 | 4 | 5 | 6 | $H_2O$ |
| SW1 | Bees | 8.6 | 4.7 | 9.9 | 9.3 | 8.5 | 8.3 | 8.8 | 11.8 | 7.8 | 13.1 | -- | -- | 0.6 |
| | Pollen | 1.5 | 1.5 | 2.2 | 2.0 | | | 2.2 | 2.5 | 1.2 | 2.6 | | | |
| SW2 | Bees | 7.6 | 6.2 | 5.3 | 12.2 | 8.5 | 8.2 | 12.0 | 10.2 | 7.8 | 9.9 | 10.1 | 8.4 | 0.2 |
| | Pollen | 1.1 | 1.8 | 2.3 | 2.8 | | | 5.0 | 1.1 | 1.5 | 1.7 | | | |
| SW3 | Bees | 8.5 | 10.7 | 7.5 | 7.9 | 10.0 | 10.9 | 6.7 | 8.9 | 14.2 | 9.1 | 11.0 | 10.6 | 0.6 |
| | Pollen | -- | -- | -- | -- | | | 2.1 | 1.7 | 0.7 | 1.3 | | | |
| S1 | Bees | 6.3 | 8.9 | 6.3 | 5.1 | 8.2 | 8.8 | 6.4 | 5.8 | 5.8 | 5.9 | 5.7 | 6.3 | 0.1 |
| | Pollen | 2.9 | 4.1 | 2.3 | 2.9 | | | 2.5 | 2.9 | 3.3 | 4.1 | | | |
| S4 | Bees | 7.2 | 6.2 | 8.1 | 9.7 | 9.7 | 9.6 | 7.8 | 8.0 | 11.4 | 12.4 | 13.6 | 12.1 | 0.6 |
| | Pollen | 0.5 | 0.6 | 0.4 | 0.9 | | | 1.7 | 2.2 | 2.8 | 1.5 | | | |
| S5 | Bees | 8.8 | 7.0 | 7.6 | 5.4 | 9.3 | 8.4 | 15.2 | 10.4 | 16.2 | 15.4 | 12.0 | 13.3 | 0.6 |
| | Pollen | 2.3 | 2.5 | 2.4 | 2.2 | | | 2.7 | 2.2 | 2.1 | 3.0 | | | |
| SE1 | Bees | 8.2 | 9.7 | 10.0 | 8.7 | 12.0 | 9.7 | 15.8 | 17.1 | 14.7 | 20.2 | 11.0 | 10.5 | 0.6 |
| | Pollen | 1.7 | 1.5 | 1.4 | 1.4 | | | 1.2 | 0.6 | 0.7 | 0.8 | | | |
| SE2 | Bees | 7.8 | 6.3 | 5.4 | 10.4 | 9.7 | 11.2 | 6.7 | 8.4 | 13.1 | 10.5 | 12.4 | 13.5 | 0.6 |
| | Pollen | 1.1 | 1.1 | 1.9 | 1.6 | | | 0.8 | 1.2 | 1.0 | 0.8 | | | |
| NE2 | Bees | 6.5 | 7.5 | 8.1 | 9.7 | 10.2 | 8.5 | 16.7 | 15.4 | 21.5 | 17.5 | 15.8 | 14.1 | 0.3 |
| | Pollen | 1.2 | 2.5 | 1.5 | 1.4 | | | 1.6 | 1.4 | 1.3 | 1.5 | | | |
| NE3 | Bees | 6.8 | 8.9 | 9.6 | 8.5 | 9.2 | 8.6 | 12.6 | 14.4 | 23.1 | 13.8 | 11.7 | 11.7 | 0.6 |
| | Pollen | 1.3 | -- | 1.9 | 2.4 | | | 1.6 | 0.8 | 1.5 | 2.8 | | | |
| NE4 | Bees | 6.6 | 3.2 | 9.1 | 6.9 | 8.1 | 7.0 | 16.2 | 16.8 | 10.4 | 11.0 | 12.5 | 12.7 | 0.6 |
| | Pollen | 1.9 | 1.4 | 1.9 | 0.6 | | | 2.1 | 1.2 | 0.9 | 3.1 | | | |
| N2 | Bees | 4.5 | 4.6 | 3.4 | 6.2 | 7.1 | 6.3 | 3.9 | 4.0 | 3.0 | -- | 1.7 | 2.3 | 0.1 |
| | Pollen | 1.5 | 2.0 | 2.1 | 1.4 | | | 2.3 | -- | -- | -- | | | |
| NE10 | Bees | 55.7 | 58.8 | 33.5 | 44.9 | 47.6 | 60.7 | 77.6 | 110.8 | 99.8 | 104.8 | 98.3 | 95.4 | 10.5 |
| | Pollen | 2.9 | 1.5 | 1.9 | 1.6 | | | 2.9 | 2.7 | 1.9 | 1.7 | | | |
| SE11 | Bees | 8.5 | 11.1 | 7.8 | -- | -- | -- | 3.8 | 5.9 | 4.0 | 3.2 | -- | -- | |
| | Pollen | 1.0 | 1.2 | 1.6 | -- | | | 0.6 | 2.8 | 3.4 | | | | |
| S9 | Bees | 10.6 | 15.2 | 20.5 | 10.5 | 16.5 | 19.2 | | | | | | | |
| | Pollen | 2.4 | 3.0 | 2.8 | 3.1 | | | | | | | | | |
| SE10 | Bees | 5.6 | 6.7 | 10.5 | 6.2 | 8.3 | 7.4 | | | | | | | |
| | Pollen | 1.8 | 2.6 | 3.6 | 2.0 | | | | | | | | | |
| SE12 | Bees | 9.8 | 10.6 | 7.2 | 8.0 | 7.8 | 9.4 | | | | | | | |
| | Pollen | 1.6 | 1.8 | 1.7 | 1.6 | | | | | | | | | |
| SE9 | Bees | 10.2 | 7.2 | 12.1 | 11.2 | 10.6 | 9.9 | 12.5 | 20.6 | -- | -- | | | |
| | Pollen | 1.3 | 1.2 | 1.3 | 1.8 | | | -- | -- | -- | -- | | | |
| NE5 | Bees | 6.2 | 6.3 | 8.4 | 7.8 | 9.3 | 6.9 | | | | | | | |
| | Pollen | 2.0 | 2.6 | 1.8 | 0.6 | | | | | | | | | |
| NE2a | Bees | 11.1 | 6.9 | 7.4 | 7.3 | 10.6 | 8.2 | | | | | | | |
| | Pollen | -- | -- | -- | -- | | | | | | | | | |
| SE6 | Bees | 10.6 | 18.3 | 8.2 | 11.4 | | | | | | | | | |
| | Pollen | -- | -- | -- | -- | | | | | | | | | |

## APPENDIX I

### TABLE I.1. BASIC STATISTICS FOR FLUORIDE IN ADULT WORKER HONEYBEES, 1975.

| | N = 79<br>Statistic | 0 Classes<br>Standard Error | Transformation Code = 0<br>Confidence Limits<br>95% | |
|---|---|---|---|---|
| Mean | 8.36 | 0.315 | 7.73 | 8.99 |
| Median | 8.00 | 0.395 | 7.21 | 8.78 |
| Variance | 7.88 | - | - | - |
| Stand. Dev. | 2.80 | - | - | - |
| Coeff. Var. | 33.55 | 2.954 | 27.67 | 39.43 |

### TABLE I.2. BASIC STATISTICS FOR FLUORIDE IN ADULT WORKER HONEYBEES, 1976.

| | N = 53<br>Statistic | 0 Classes<br>Standard Error | Transformation Code = 0<br>Confidence Limits<br>95% | |
|---|---|---|---|---|
| Mean | 11.27 | 0.692 | 9.88 | 12.67 |
| Median | 11.00 | 0.867 | 9.24 | 12.74 |
| Variance | 25.39 | - | - | - |
| Stand. Dev. | 5.03 | - | - | - |
| Coeff. Var. | 44.67 | 5.133 | 34.36 | 54.99 |

F = 3.2216, which is highly significant.

The exact probability of F = 3.2216 with 78 and 52 D.F. is 0.00003; therefore the variances for 1975, 1976 are unequal. $(H_i:\sigma_1^2 \neq \sigma_2^2)$.

APPENDIX I (continued)

TABLE I.3. PPM FLUORIDE IN ADULT WORKER HONEYBEES, 1975 AND 1976, TWO LEVEL NESTED ANOVA.

Anova Table

| Level | SS | DF | MS | FS |
|---|---|---|---|---|
| 2 (years) | 269.004 | 1 | 269.003 | 6.3928* |
| 1 (sites) | 1339.043 | 32 | 1.845 | 6.8753** |
| | | 31.9 | 42.079 | |
| 0 (hives/site) | 596.458 | 98 | 6.086 | |

VARIANCE COMPONENTS

| Level | Variance Component | Percent |
|---|---|---|
| 2 | 3.577 | 18.947 |
| 1 | 9.215 | 48.813 |
| 0 | 6.086 | 32.238 |

*Exact probability of FS = 6.3928; DF 1, 31.9; is 0.0125.

**Exact probability of FS = 6.8753; DF 32, 98; is less than 0.001.

TABLE I.4. BASIC STATISTICS FOR FLUORIDE IN ADULT WORKER HONEYBEES. 1975 VS. 1976

(Approximate test of equality of means when variances are heterogenous.)

| Sample | Number | Mean | Variance |
|---|---|---|---|
| (1975) | 1 | 8.36 | 7.883 |
| (1976) | 2 | 11.27 | 25.396 |

$F_s^1 = 14.648$

DF = 1 and 73.8

t = 3.83

$F_s^1$ is greater than $F_{.001[1,\ 73.8]} = 11.9$; we reject the null hypothesis that samples were drawn from populations with equal mean fluoride content.

The exact probability of F = 14.648 with (1, 73.8) DF is 0.00051.

## SECTION 6

# THE EFFECTS OF COAL-FIRED POWER PLANT EMISSIONS ON VERTEBRATE ANIMALS IN SOUTHEASTERN MONTANA (A REPORT OF PROGRESS)

by

R. A. Lewis, M. L. Morton, M. D. Kern, J. D. Chilgren, and E. M. Preston

## INTRODUCTION

Vertebrate responses to air pollution vary seasonally with the quality and quantity of ecosystem resources, with the sex, age, or physiological state of the organism, and with secondary stressors, such as disease, competitive interactions, or other pollutants. Hence, field work in this project has emphasized the description and evaluation of annual and life cycles and the elucidation of mechanisms that regulate these cycles.

The preoperational (baseline) phase of the investigation is nearing completion, although analyses of this work will continue for some time. Major objectives for this phase of the study are to:

1. Measure and predict changes in population structure and/or dynamics of grassland birds and small mammals as a function of annual, seasonal, and life cycles as well as other environmental information including biotic interactions and physical factors that influence the structural and dynamic processes under study;
2. assess the specific effects of air pollution, if possible, upon avian and mammalian population structure and dynamics as well as upon specific organ systems, and
3. evaluate selected physiological, biochemical, and behavioral functions that may have potential for sensitive assay of pollution challenge. Hopefully, low levels of pollution stress will be identified before serious or irreversible effects occur.

Although considerably less substantial than that for birds, the baseline work on mammals is more complete at this time and is therefore emphasized in this synopsis. The preoperational components of the study will be completed for mammals in 1977, but not until 1978 for birds. The bulk of the remaining bird work will be performed under grant by appropriate investigators. The major components are:

1. Reproductive and developmental biology;

2. measures of condition, physiologic stress, homeostasis, and adaptation;

3. population biology, and

4. histological cycles of organ-systems of potential or probable concern.

Field experiments, now completed, on the effects of sulfur dioxide on the population movements and physiology of small mammals will be treated in a subsequent report.

Avian species being studied are the Western Meadowlark (_Sturnella neglecta_), Mourning Dove (_Zenaidura macroura_), Lark Bunting (_Calamospiza melanocorys_), Vesper Sparrow (_Pooecetes gramineus_), and Lark Sparrow (_Chondestes grammacus_). The mammals of greatest importance are the Deer Mouse (_Peromyscus maniculatus_) and Prairie Vole (_Microtus ochrogaster_).

The reproductive and developmental portions of the study emphasize growth and development of the young, bioenergetics, productivity, and the regulation of reproductive processes and molt. The physiological component treats those functions that reflect condition and vigor of the species and their stress responses. Population parameters, such as abundance and dispersion of species, sex, and age ratios, and some of the mechanisms that effect population adjustments are also of concern. Finally, the study of histological cycles is designed to help interpret mechanisms at all levels and to identify direct and indirect (e.g., intercurrent disease) effects of pollution.

## MAMMALS

### INTRODUCTION

The study of small mammals is largely confined to the mice and voles (Cricetidae). These are the most abundantly distributed and easily trapped mammals in southeastern Montana. Some information is also being gathered on members of the families Heteromyidae (including pocket mice), and Sciuridae (squirrels, chipmunks, etc.).

The use of small mammals as indicator species of air pollution is not new (e.g., Kay, 1974), but it has not received much attention. Although the impact of small mammals on the character of grassland ecosystems is reasonably well documented (Golley _et al_., 1975), their potential importance with respect to ecosystem responses to air pollution has rarely been considered. Small mammals in the Northern Great Plains contribute to maintenance of the health and vigor of the grasslands. Mechanisms include the following:

1. Grazing, within limits, promotes the growth of plants. Thus, grazing by rodents may have a significant effect on the productivity of the range.

2. Burrows of fossorial mammals may increase water storage and retention in the soil.

3. Burrowing increases transport of minerals from deeper to upper soil layers and may increase the rate of decomposition of organic matter.

4. Feces may enrich soil biota.

5. Specific effects depend in large part upon habits and population structure of the predominant species in a community. For example, some species consume insects (many of which are harmful from man's point of view) or weed seeds, whereas others can damage agricultural crops.

6. Rodents are important food of many avian and mammalian predators.

Many changes in rodent population structure would be expected to have an appreciable impact upon grasslands.

## STUDY AREAS AND METHODS

The information on small mammals presented in this report was gathered at selected sites east or southeast of Colstrip, Montana, between the spring of 1974 and the fall of 1976. Investigators were in the field each month. Thus seasonal changes as well as instantaneous evaluations of many functions were assessed. In 1976 only the McRae site remained under intensive investigation.

Five locations were selected, surveyed, and staked in mixed sagebrush-grassland habitat in the spring of 1974. Four of these sites (Kluver North, Kluver East, Kluver West, and McRae's) were chosen because of their proximity to exclosures constructed for grassland studies (see other reports, this volume). The remaining site was located on Pony Creek about one km west of Garfield's ranch house. Square grids 150 m on a side (2.25 ha) were constructed. Trapping stations were 15 m apart (121 stations per grid). Each month during the 1974 and 1975 field seasons, Sherman live traps (7.6 x 7.6 x 25.4 cm) were baited with rolled oats for three or four nights with free entry and exit (prebaiting). This was followed by a one-day capture, mark, and release (CMR) trapping session. In 1976 the traps were prebaited for two nights followed by three successive sessions of capture, mark, and release. This procedure was repeated at three-week intervals. Traps were set at dusk and picked up at dawn. In cold weather tissue paper for nest material and large quantities of bait were provided to enhance survival. The toes (no more than one per foot) of trapped mice were clipped to permit individual identification. Mice were sexed, aged, and weighed to the nearest 0.1 g on a pan balance. They were also examined externally for signs of sexual activity and pelage changes. All were released at their station of capture.

Specimens were collected at weekly intervals in live traps and transported to the laboratory at Fort Howes Ranger Station. Linear dimensions (body, ear to notch, hind foot, and tail) were measured with precision calipers. Following ether anesthesia, two blood samples were taken from the orbital sinus in heparinized capillary tubes. Hematocrit was determined from these samples following high-speed centrifugation for 10 minutes. Hemoglobin concentration was measured spectrophotometrically by the cyanmethemoglobin technique, and plasma protein concentration was measured with a diffraction meter. Heart, lungs, kidneys, adrenals, spleen, liver, and reproductive tracts were removed and fixed in Bouin's solution or 10% neutral formalin, and after one week weighed to the nearest 0.2 mg on a torsion balance. After weighing, tissues fixed in Bouin's were transferred to 70% ethanol. Reproductive tracts collected in September and October 1975 were supplied by Don Dodge from C. Gordon's laboratory. Gonadal weights given are always those of the paired glands.

It was noted that reproductive readiness, large testes, or pregnancy were not observed in P. maniculatus weighing less than 14 g. Therefore, the term "immature" is applied to animals below 14 g body weight and "adult" to those 14 g or heavier.

## REPRODUCTIVE AND DEVELOPMENTAL BIOLOGY

The reproductive and developmental portions of the study emphasize description of the annual reproductive cycles of a small set of indigenous species. The evaluations are not complete at this time.

### Females

Pregnant deer mice occurred in the samples from early spring 1974 through late summer 1975 (Table 6.1). The first two females trapped in March 1975 were pregnant. Embryo lengths in utero were 60 mm and 110 mm, the latter probably near term. In 1975 the proportion of pregnant females decreased in July and August and in September only one of 20 had embryos. None of the 11 adults trapped in October were pregnant. In September 1974, however, one of three adults was pregnant and in late October four of five adults were pregnant, although none of five taken in early October were pregnant. A resurgence of breeding in October 1974 may have been related to unusually wet weather during which there was new growth of grasses. Precipitation in October 1974 was more than double that of the long term average.

Litter sizes of all species, based on dissections of pregnant females, are presented in Table 6.2. In P. maniculatus litter size (1975) based on counts of uterine scars was not significantly higher ($0.10 < P < 0.20$) than counts of visible embryos. Similarly, litter size did not vary significantly throughout the season.

Litter sizes between 1975 and 1976 differed significantly for both the deer mouse ($P < 0.05$) and the prairie vole. Data for the voles, however, is based upon a small sample size and might merely reflect variation in area-specific populations. Nearly all pregnant voles in 1976 were obtained from one region of about 250 $m^2$. One major difficulty in the study of the prairie vole is its erratic distribution.

A chi-square test revealed no difference in distribution of embryos between the two uterine horns ($P > 0.98$) in P. maniculatus.

The weight of the reproductive tract of female mice changes seasonally in association with the production of young. Thus, in P. maniculatus there was about a four-fold difference between monthly mean ovarian weight in winter and

TABLE 6.1. PERCENT OF PREGNANT FEMALES IN SAMPLES OF *Peromyscus maniculatus* COLLECTED NEAR COLSTRIP.

| Month | | Number in Sample | Percent Pregnant |
|---|---|---|---|
| July | 1974 | 4 | 75.0 |
| August | | 8 | 62.5 |
| September | | 14 | 28.6 |
| October | | 10 | 40.0 |
| November | | 8 | 0.0 |
| December | | 10 | 0.0 |
| January | 1975 | 3 | 0.0 |
| March | | 2 | 100.0 |
| April | | 9 | 77.8 |
| May | | 6 | 66.7 |
| June | | 8 | 75.0 |
| July | | 9 | 33.3 |
| August | | 10 | 40.0 |
| September | | 20 | 5.0 |
| October | | 11 | 0.0 |
| April | 1976 | 18 | 33.3 |
| May | | 4 | 25.0 |
| June | | 13 | 46.2 |
| July | | 7 | 57.1 |
| August | | 10 | 40.0 |
| September | | 3 | 33.3 |

TABLE 6.2. LITTER SIZE IN MICE COLLECTED NEAR COLSTRIP.

| Species | Size determinant | N | Mean | SD | Range |
|---|---|---|---|---|---|
| *Peromyscus maniculatus* | 1975 uterine scars | 9 | 5.33 | 0.87 | 4-6 |
| | 1976 uterine scars | 2 | 6.50 | -- | 6.7 |
| | 1975 embryos | 42 | 4.95 | 0.99 | 3.7 |
| | 1976 embryos | 22 | 5.68 | 0.72 | 4-7 |
| | 1975 total | 24 | 5.75 | 0.74 | 4-7 |
| *Microtus ochrogaster* | 1975 embryos | 11 | 3.00 | 0.85 | 2-4 |
| | 1976 embryos | 6 | 5.00 | 0.89 | 4-6 |
| *Perognathus fasciatus* | 1975 embryos | 1 | 6.00 | -- | --- |
| | 1976 embryos | 2 | 5.00 | -- | --- |
| *Reithrodontomys megalotis* | 1975 + 1976 | 2 | 4.00 | -- | --- |

summer and a 50-fold difference in uterine weight (Figure 6.1). The largest average ovarian weights were recorded in April and May and the lowest in November, December, and January. Mean uterine weight in October 1975 was strongly biased by one female with seven near-term implanted embryos, whose uterus weighed 11.9 g. Mean uterine weight of the other 19 females in the sample was only 29.8 mg.

Ovarian weight in P. maniculatus varied seasonally and in proportion to body weight ($P < 0.005$). The least squares regression line of ovarian weight on body weight in pregnant females was $Y = 3.13 \pm 0.69X$ (standard error of estimate of Y on $X = 3.72$; $r = 0.574$).

M. ochrogaster is reproductively active throughout the year except in mid-winter (see Table 6.3). A pregnant female and a male with scrotal testes taken on December 5 had just reached sexual maturity, as judged by body weight, and were probably engaged in their first breeding effort. The data do not permit accurate determination of the annual reproductive period in M. ochrogaster, but reproductive tracts were definitely reduced in size during winter months and only one male with scrotal testes was collected from November through January (Table 6.3).

## Males

There is a marked seasonal variation in testicular weight of P. maniculatus (Figure 6.2). Maximum weight and presumably peak functional status were maintained in the samples from March through August.

A cycle in seminal vesicle weight paralleled that of the testes during phases of peak and decreasing testicular size but lagged during testicular recrudescence. This expected relationship illustrates the trophic effect of testosterone on sexual accessory organs (McKeever, 1964a, b; Cinq-Mars and Brown, 1969; Chapman, 1972). The decision to assign adult status to P. maniculatus with a body weight of 14 g or more was based upon visual inspection of reproductive tracts. Flake (1974) found minimum weight of pregnant P. maniculatus to be 18 g, but investigators in this research found it to be at least as low as 14.3 g. Individual males of intermediate size exhibited a seasonal change in tendency to reach sexual maturity. Males in May and June tended to have larger testes and seminal vesicles than individuals of comparable size later in the reproductive season in July and August (Figure 6.3). Testicular weights differed significantly ($P < 0.05$) among all body weight groups except 14.0 to 14.9 g, as did seminal vesicle weights ($P < 0.01$) in animals weighing 16.0 g or more.

The breeding season in eastern Montana has not been previously documented for any species of mouse, but in Colorado pregnant deer mice (P. maniculatus) were recorded primarily from April through September (Lechleitner, 1969), and in Wyoming from April through August (Brown, 1966). Data show that the reproductive period of deer mice in the Colstrip area extends from mid-March to mid-September.

The distinct breeding cycle in P. maniculatus results in pronounced and regular variation in the number and proportions of immatures captured (Figures

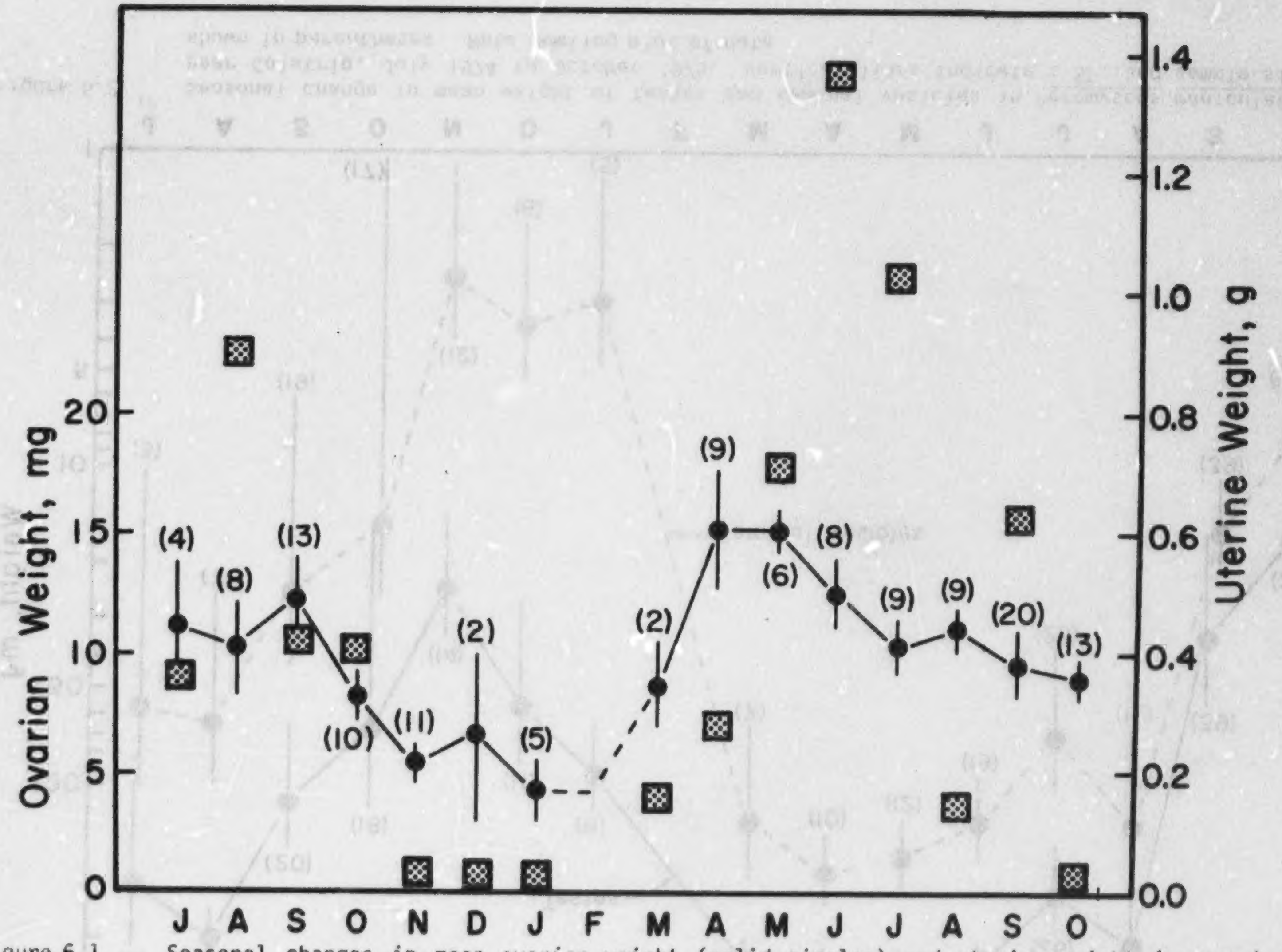

Figure 6.1. Seasonal changes in mean ovarian weight (solid circles) and uterine weight (squares) in Peromyscus maniculatus near Colstrip, Montana, July 1974 to October 1975. Vertical lines indicated ± 2 SE for ovarian weight. Sample size for both organs shown in parentheses.

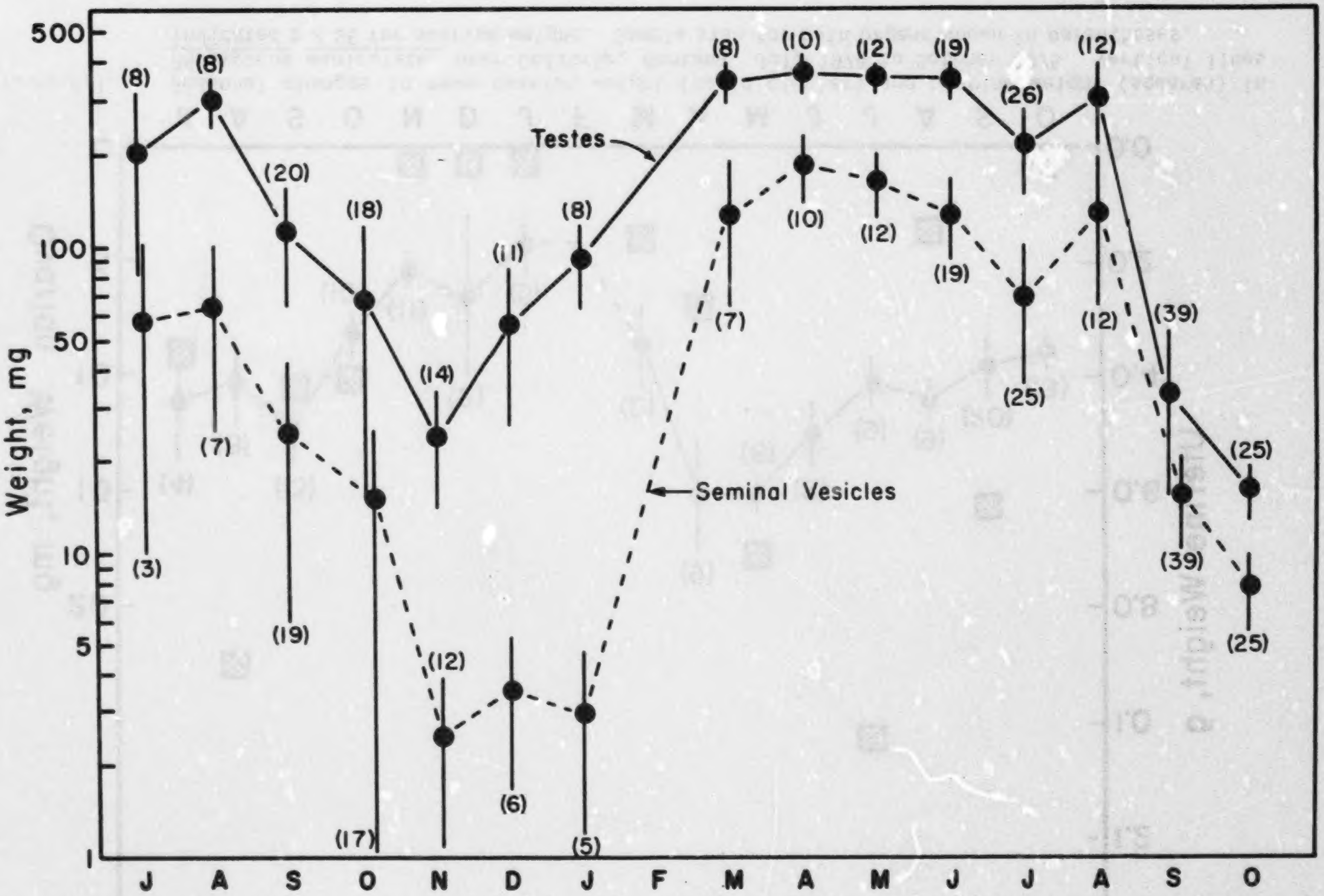

Figure 6.2. Seasonal change in mean weight of testes and seminal vesicles in Peromyscus maniculatus near Colstrip, July 1974 to October 1975. Vertical lines indicate ± SE, and sample size shown in parentheses. Note semilog plot of data.

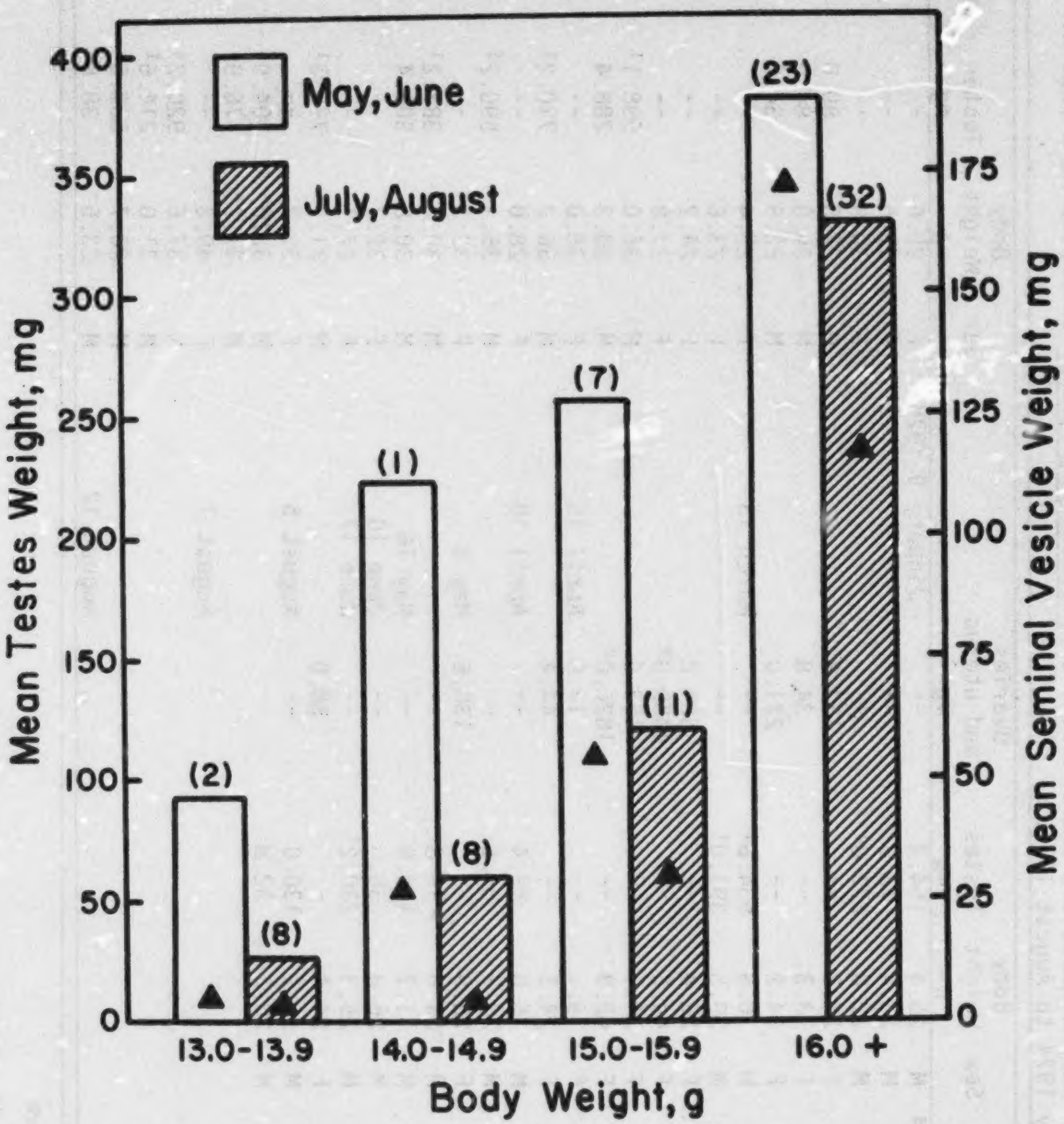

Figure 6.3. Early (May, June) and late (July, August) summer differences in testes weight (histograms) and seminal vesicles (triangles) in young Peromyscus maniculatus. Sample size shown in parentheses.

TABLE 6.3. BODY AND REPRODUCTIVE ORGAN WEIGHT IN Microtus ochrogaster COLLECTED NEAR COLSTRIP, MONTANA July 1974 to August 1975.

| | | Sex | Body Weight g | Testes mg | Ovaries and uterus mg | | Sex | Body Weight g | Testes mg | Ovaries and uterus mg |
|---|---|---|---|---|---|---|---|---|---|---|
| July 28 | 1974 | M | 25.4 | 154.2 | -- | January 9 1975 | F | 25.6 | -- | 48.0 |
| August 9 | | M | 23.4 | 73.8 | -- | | F | 29.5 | -- | 58.0 |
| | | M | 28.8 | 273.8[1] | -- | | F | 30.9 | -- | 84.8 |
| August 20 | | F | 28.3 | -- | 133.8 | | M | 22.8 | 80.0 | -- |
| | | F | 13.3 | -- | 34.8 | | M | 34.0 | 84.0 | -- |
| | | F | 34.2 | -- | 221.0 | | M | 22.9 | 34.0 | -- |
| | | M | 38.9 | 534.6[1] | -- | March 13 | F | 25.4 | -- | 94.6 |
| | | M | 30.5 | 391.0[1] | -- | | F | 23.6 | -- | 306.7[2] |
| September 3 | | F | 28.4 | -- | 384.0 | | F | 24.2 | -- | 72.0 |
| | | F | 44.5 | -- | 4450.0[2] | | F | 27.8 | -- | 44.8 |
| | | F | 34.1 | -- | 453.6 | | M | 36.0 | 598.1[1] | -- |
| | | F | 42.3 | -- | 1820.0[2] | | M | 28.3 | 288.4 | -- |
| November 27 | | F | 19.1 | -- | 14.8 | April 15 | F | 38.0 | -- | 1080.0[2] |
| | | F | 24.5 | -- | 44.4 | | M | 36.2 | 710.2[1] | -- |
| | | M | 26.0 | 82.4 | -- | April 18 | F | 28.0 | -- | 200.2 |
| | | M | 23.7 | 66.8 | -- | | M | 35.1 | 590.2[1] | -- |
| December 5 | | F | 28.9 | -- | 130.6 | May 6 | F | 33.3 | -- | 388.2[2] |
| | | M | 21.0 | 18.8 | -- | | M | 30.3 | 387.2[1] | -- |
| | | M | 31.7 | 124.8 | -- | May 16 | M | 30.8 | 505.4 | -- |
| | | M | 26.8 | 38.0 | -- | June 10 | F | 32.7 | -- | 408.2[2] |
| | | M | 25.3 | 230.2[1] | -- | June 17 | F | 27.1 | -- | 187.6[2] |
| December 17 | | F | 24.4 | -- | 58.0 | | M | 31.2 | 780.3[1] | -- |
| | | M | 36.2 | 130.0 | -- | August 5 | F | 39.2 | -- | 207.2[2] |
| | | M | 20.3 | 32.8 | -- | | M | 32.0 | 104.9[1] | -- |
| | | | | | | | M | 37.2 | 76.9[1] | -- |
| | | | | | | August 7 | F | 40.3 | -- | 266.0[2] |
| | | | | | | | M | 37.6 | 920.2[1] | -- |
| | | | | | | | M | 31.0 | 214.6[1] | -- |
| | | | | | | | M | 20.7 | 362.0[1] | -- |
| | | | | | | August 12 | M | 23.6 | 38.6 | |

[1] Testes in scrotum

[2] Embryos visible in uterus

6.4 and 6.5). All deer mice captured in March were adults, but one caught on April 18 was 12 g. In 1976 the smallest immatures captured in April were 7.0, 8.5, and 11.9 g captured on April 9, 13, and 23, respectively. The proportion of immatures in the trapped sample increased thereafter, peaking in October of 1974 and September or thereafter in 1976. Immatures captured in mid-winter were undoubtedly from the last litters of autumn.

During much of the reproductive period females are heavier than males (Figure 6.6). This weight increase was undoubtedly associated with pregnancy. No differences in linear body dimensions between adult males and adult non-pregnant females were found during this time (Tables 6.4 and 6.5). When all animals sampled for the duration of the study were compared, however, tail length was significantly greater in females ($t = 1.90$, $P < 0.05$) whereas hind foot length was considerably greater in males ($t = 3.09$, $P < 0.005$). The sexes did not differ in body length ($t = 0.98$, $P < 0.10$) or in ear length ($t = 0.87$, $P < 0.10$).

## MEASURES OF CONDITION, PHYSIOLOGICAL STRESS, HOMEOSTASIS, AND ADAPTATION

The blood vascular system of mammals is complex but well characterized. Blood carries erythrocytes that are involved in oxygen transport, leucocytes that mediate responses to insult, hormones, enzymes and other proteins, and a host of other chemicals that are characteristic of a given functional state. Some of the most easily assessed characteristics include hematocrit (percentage of packed red blood cells occupied by a given volume of whole blood), hemoglobin content, erythrocyte count, and plasma protein analysis. The first three are highly regulated in vertebrate vascular systems. Plasma protein varies more in relation to other factors such as blood osmotic pressure and nutritional state. Hematocrit, blood hemoglobin content, and plasma protein concentration were measured in rodents trapped in all three years of the study. In addition, serum acetylcholinesterase assays have been performed on blood samples taken in 1976.

The hematocrit ratio and hemoglobin concentration of *M. ochrogaster* were higher in winter (December and January) than in summer (April to September), whereas plasma protein concentration was higher in summer (Table 6.6). Blood data for *P. maniculatus* were analyzed by sex in both winter (November to January) and summer (April to September) (Table 6.7). As in *M. ochrogaster*, hematocrit tended to be higher in winter than in summer. Unlike *M. ochrogaster*, however, hemoglobin of males was higher in summer than in winter (Table 6.7). The data indicate that mean corpuscular hemoglobin concentration (MCHC) decreased during winter (Table 6.8). There was a statistically significant decrease in plasma protein concentration (PPC) during winter in both male and female *P. maniculatus* (Table 6.7).

In 1976 seasonal changes in blood functions were not determined since the sampling period spanned only six months (April to September). However, sufficient samples were taken to determine further sexual differences in hematocrit, hemoglobin, and plasma protein for both the deer mouse and prairie vole (Tables 6.9, 6.10, 6.11). In the vole, males had significantly higher values of hematocrit and hemoglobin than females (Table 6.9). There was also a tendency for higher plasma protein readings in males.

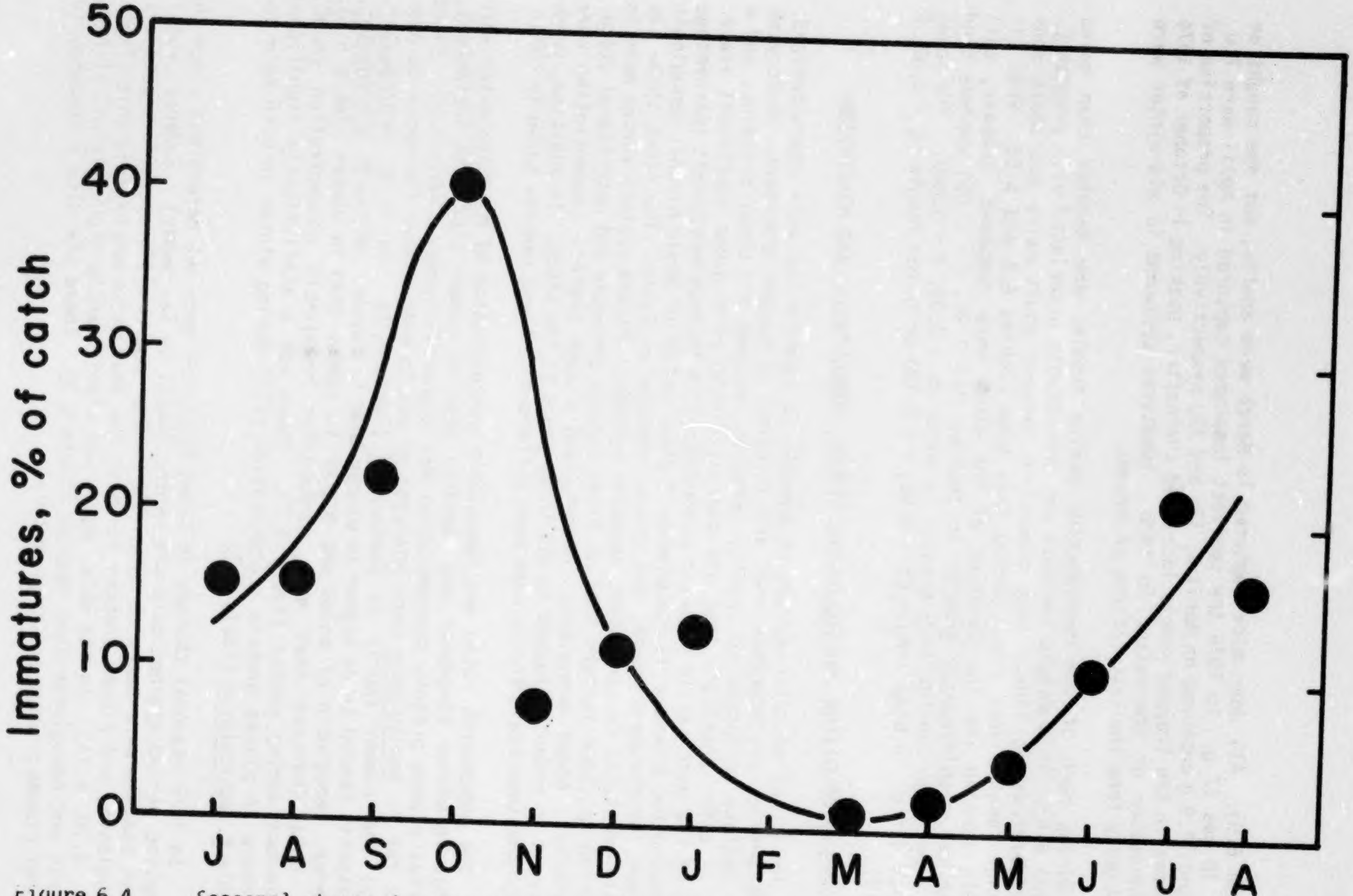

Figure 6.4. Seasonal change in percent of *Peromyscus maniculatus* captured as immatures (body weight less than 14 g) near Colstrip, July 1974 to August 1975.

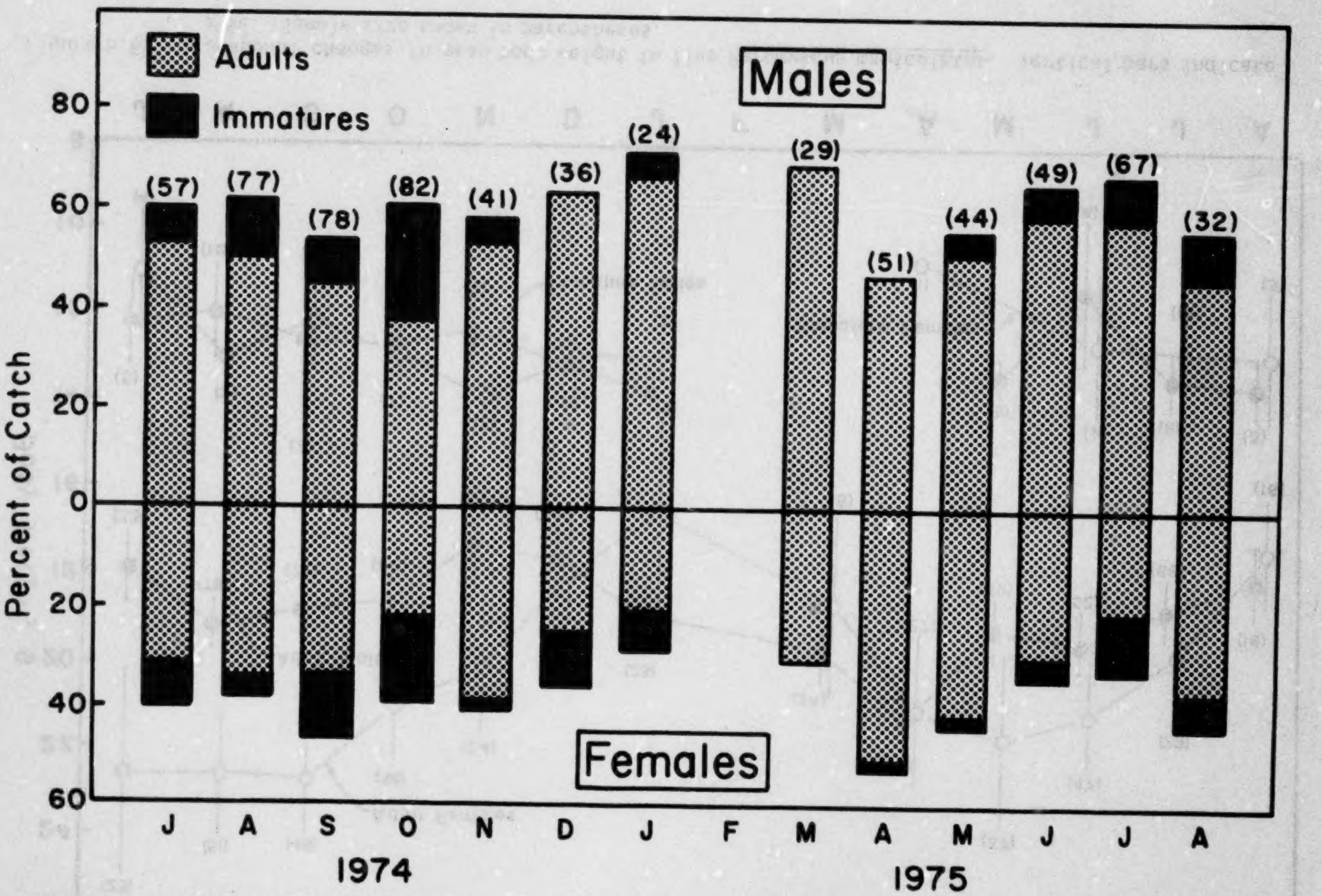

Figure 6.5. Seasonal change in sex and age of Peromyscus maniculatus captured near Colstrip, July 1974 to August 1975. Sample size shown in parentheses.

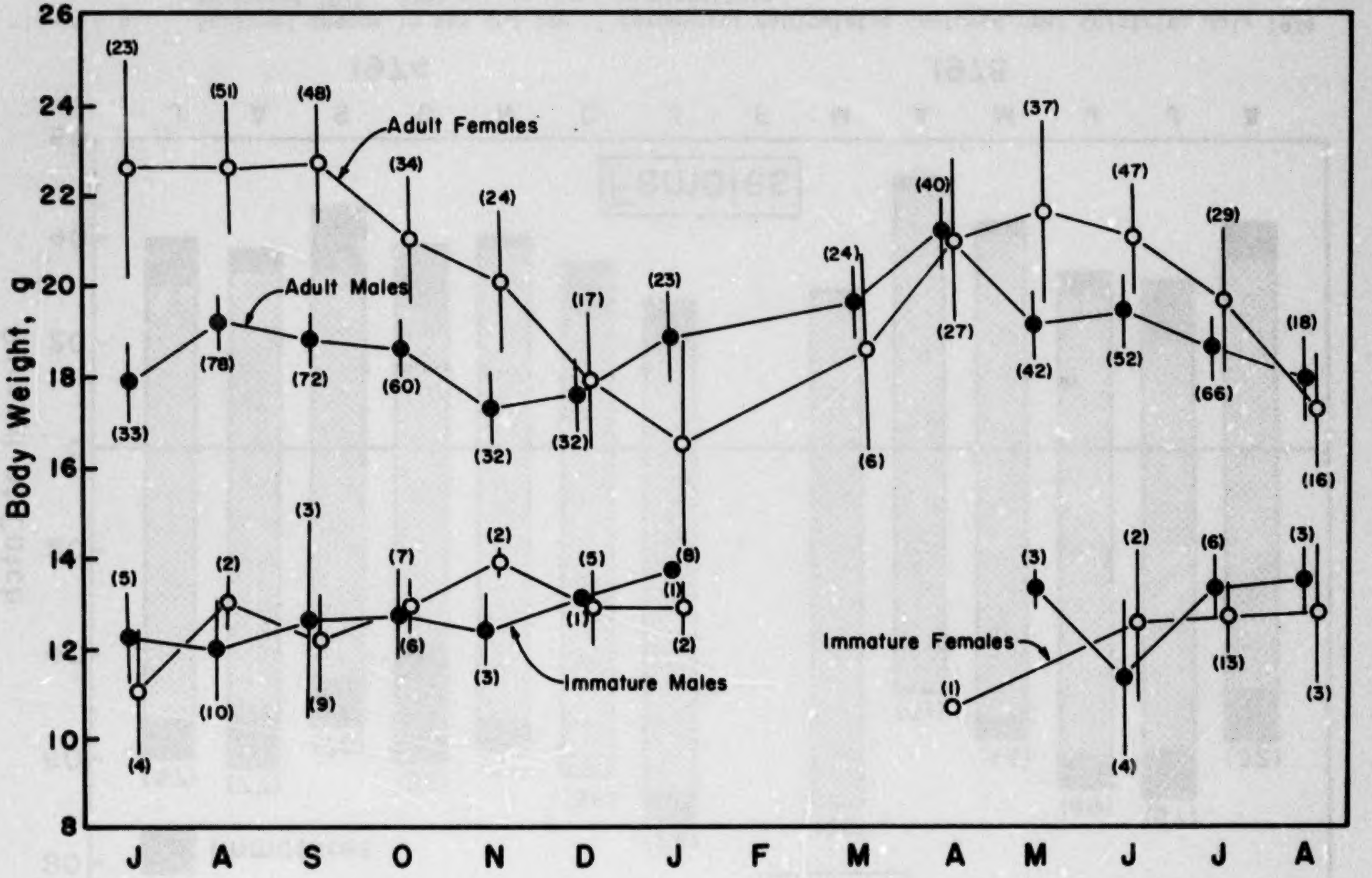

Figure 6.6. Seasonal changes in mean body weight in live Peromyscus maniculatus. Vertical bars indicate ± SE. Sample size shown in parentheses.

TABLE 6.4. SEASONAL CHANGES IN LINEAR MEASUREMENTS IN MILLIMETERS OF ADULT, NON-PREGNANT FEMALE *Peromyscus maniculatus* CAPTURED NEAR COLSTRIP, MONTANA, July 1974 to August 1975.

| | Body Length | | | Tail Length | | | Hindfoot Length | | | Ear Length | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | S.D. | (N) | Mean | S.D. | (N) | Mean | S.D. | (N) | Mean | S.D. | (N) |
| July 1974 | 89.8 | 3.2 | 12 | 63.7 | 4.0 | 12 | 19.0 | 0.9 | 12 | 16.0 | 1.2 | 12 |
| August | 89.9 | 6.4 | 37 | 63.9 | 4.1 | 37 | 18.4 | 1.2 | 37 | 15.5 | 1.2 | 38 |
| September | 84.8 | 4.9 | 33 | 61.5 | 5.4 | 33 | 18.6 | 1.2 | 33 | 15.5 | 1.4 | 33 |
| October | 81.0 | 8.1 | 6 | 56.3 | 6.2 | 6 | 18.5 | 1.0 | 6 | 15.8 | 1.3 | 6 |
| November | 85.1 | 5.8 | 11 | 57.0 | 4.9 | 11 | 18.5 | 0.7 | 11 | 16.7 | 1.2 | 11 |
| December | 79.7 | 3.1 | 7 | 56.0 | 4.2 | 7 | 18.7 | 0.5 | 7 | 15.3 | 0.5 | 7 |
| January 1975 | 70.0 | 0.0 | 3 | 48.0 | 2.0 | 3 | 17.7 | 0.6 | 3 | 14.3 | 0.3 | 3 |
| April | 88.7 | 5.1 | 6 | 58.2 | 4.3 | 5 | 18.7 | 0.5 | 6 | 16.3 | 1.2 | 6 |
| June | 83.0 | ---- | 2 | 65.0 | 5.7 | 2 | 19.5 | --- | 2 | 17.0 | --- | 2 |
| July | 88.7 | 11.5 | 9 | 58.7 | 8.6 | 9 | 19.6 | 0.5 | 9 | 16.8 | 1.6 | 9 |
| August | 86.5 | 3.6 | 5 | 58.0 | 3.0 | 5 | 19.4 | 0.5 | 5 | 16.6 | 0.7 | 5 |
| Total Sample | 86.4 | 7.1 | 131 | 60.9 | 5.9 | 130 | 18.7 | 1.0 | 131 | 15.8 | 1.2 | 132 |

TABLE 6.5. SEASONAL CHANGES IN LINEAR MEASUREMENT IN MILLIMETERS OF ADULT MALE *Peromyscus* CAPTURED NEAR COLSTRIP, MONTANA, July 1974 to August 1975.

| | Body Length | | | Tail Length | | | Hindfoot Length | | | Ear Length | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | S.D. | (N) | Mean | S.D. | (N) | Mean | S.D. | (N) | Mean | S.D. | (N) |
| July 1974 | 87.6 | 4.2 | 22 | 62.0 | 4.2 | 22 | 19.4 | 0.9 | 22 | 16.2 | 1.3 | 22 |
| August | 90.6 | 5.0 | 65 | 63.1 | 4.2 | 65 | 18.6 | 1.3 | 65 | 15.8 | 1.1 | 65 |
| September | 82.0 | 5.5 | 61 | 60.4 | 4.7 | 61 | 18.8 | 1.1 | 61 | 15.2 | 1.2 | 61 |
| October | 85.2 | 5.9 | 17 | 58.2 | 5.9 | 16 | 18.8 | 0.8 | 17 | 16.3 | 1.3 | 17 |
| November | 83.2 | 4.4 | 15 | 53.9 | 5.9 | 15 | 18.8 | 0.9 | 15 | 15.7 | 0.9 | 15 |
| December | 79.4 | 4.9 | 11 | 56.3 | 7.4 | 11 | 19.0 | 1.0 | 11 | 15.5 | 1.4 | 11 |
| January 1975 | 73.4 | 5.8 | 8 | 52.1 | 5.5 | 8 | 18.5 | 0.9 | 8 | 14.6 | 0.7 | 8 |
| March | 84.7 | 6.3 | 7 | 57.3 | 4.8 | 7 | 20.9 | 3.2 | 7 | 15.2 | 1.3 | 7 |
| April | 88.1 | 7.0 | 10 | 58.7 | 5.1 | 10 | 18.7 | 0.8 | 10 | 15.9 | 1.1 | 10 |
| May | 83.0 | 3.4 | 10 | 58.4 | 4.7 | 10 | 19.5 | 0.6 | 10 | 16.6 | 0.6 | 10 |
| June | 85.8 | 5.3 | 16 | 61.3 | 4.9 | 16 | 19.5 | 0.9 | 16 | 17.1 | 0.8 | 16 |
| July | 87.1 | 6.0 | 26 | 57.9 | 3.8 | 26 | 19.7 | 0.7 | 26 | 16.9 | 0.8 | 26 |
| August | 89.9 | 3.7 | 12 | 58.1 | 4.2 | 12 | 19.4 | 0.5 | 12 | 16.9 | 0.9 | 12 |
| Total Sample | 85.7 | 6.6 | 280 | 59.8 | 5.5 | 279 | 19.0 | 1.2 | 280 | 15.9 | 1.3 | 280 |

TABLE 6.6. HEMATOLOGICAL VALUES FOR *Microtus ochrogaster* COLLECTED NEAR COLSTRIP. July 1974 to August 1975.

| | N | Mean | S.D. | t | P |
|---|---|---|---|---|---|
| Hematocrit, (%) | | | | | |
| Summer | 20 | 49.80 | 4.42 | -- | -- |
| Winter | 13 | 52.23 | 4.90 | 1.65 | >0.05 |
| Hemoglobin, (g%) | | | | | |
| Summer | 13 | 15.42 | 1.26 | -- | -- |
| Winter | 6 | 17.20 | 1.18 | 2.97 | <0.005 |
| Plasma protein, (g%) | | | | | |
| Summer | 20 | 6.22 | 0.44 | -- | -- |
| Winter | 13 | 5.97 | 0.43 | 1.67 | >0.05 |

TABLE 6.7. HEMATOLOGICAL VALUES FOR *Peromysus maniculatus* COLLECTED NEAR COLSTRIP. July 1974 to August 1975.

| | N | Mean | S.D. | t | P |
|---|---|---|---|---|---|
| Hematocrit, (%) | | | | | |
| Summer females | 60 | 48.48 | 3.44 | -- | -- |
| Winter females | 18 | 50.06 | 5.58 | 1.14 | >0.10 |
| Summer males | 114 | 49.88 | 4.42 | -- | -- |
| Winter males | 25 | 52.24 | 3.07 | 3.19 | <0.005 |
| All females | 78 | 48.85 | 4.05 | -- | -- |
| All males | 139 | 50.30 | 4.30 | 2.50 | <0.01 |
| Hemoglobin, (g%) | | | | | |
| Summer females | 37 | 15.85 | 1.31 | -- | -- |
| Winter females | 5 | 14.45 | 2.64 | 1.17 | >0.10 |
| Summer males | 75 | 15.87 | 1.40 | -- | -- |
| Winter males | 6 | 15.29 | 0.46 | 2.32 | <0.025 |
| All females | 42 | 15.68 | 1.55 | -- | -- |
| All males | 81 | 15.82 | 1.36 | 0.50 | >0.30 |
| Plasma Protein, (g%) | | | | | |
| Summer females | 43 | 6.39 | 0.75 | -- | -- |
| Winter females | 18 | 6.03 | 0.60 | 1.98 | <0.05 |
| Summer males | 86 | 6.39 | 0.56 | -- | -- |
| Winter males | 22 | 6.17 | 0.46 | 1.90 | <0.05 |
| All females | 61 | 6.28 | 0.68 | -- | -- |
| All males | 108 | 6.35 | 0.52 | 0.70 | >0.20 |

TABLE 6.8. MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION IN RODENTS COLLECTED NEAR COLSTRIP. July 1974 to August 1975.

| Species and Season | MCHC[1] |
|---|---|
| *Microtus ochrogaster* | |
| Summer | 31.0 |
| Winter | 32.9 |
| *Peromyscus maniculatus* | |
| Summer females | 32.7 |
| Winter females | 28.9 |
| Summer males | 31.8 |
| Winter males | 29.3 |

$$MCHC^1 = \frac{100 \text{ (mean hemoglobin concentration)}}{\text{mean hematocrit}}, \text{ as computed}$$

TABLE 6.9. HEMATOLOGICAL VALUES FOR *Microtus ochrogaster* COLLECTED NEAR COLSTRIP. April to September 1976.

| Function | Sex | N | Mean | SD | *t* | P |
|---|---|---|---|---|---|---|
| Hematocrit (%) | M | 11 | 51.50 | 3.04 | 2.54 | <0.02 |
| | F | 12 | 48.73 | 2.16 | | |
| Hemoglobin (g %) | M | 11 | 16.89 | 1.06 | 2.45 | <0.05 |
| | F | 10 | 15.89 | 0.77 | | |
| Plasma protein (g %) | M | 11 | 6.15 | 0.49 | 1.18 | <0.40, NS |
| | F | 12 | 5.57 | 1.80 | | |

TABLE 6.10. HEMATOLOGICAL VALUE FOR *Peromyscus maniculatus* COLLECTED NEAR COLSTRIP. April to September 1976.

| Function | Sex | N | Mean | SD | *t* | P |
|---|---|---|---|---|---|---|
| Hematocrit (%) | M | 93 | 50.80 | 3.69 | 4.34 | <0.001 |
| | F | 49 | 48.12 | 3.10 | | |
| Hemoglobin (g %) | M | 79 | 16.93 | 1.36 | 3.19 | <0.01 |
| | F | 37 | 16.08 | 1.26 | | |
| Plasma protein (g %) | M | 106 | 6.20 | 1.06 | -- | NS |
| | F | 55 | 6.21 | 0.72 | | |

TABLE 6.11. HEMATOLOGICAL VALUES OF *Perognathus fasciatus* COLLECTED NEAR COLSTRIP. April to September 1976.

| Function | N | Mean | SD |
|---|---|---|---|
| Hematocrit (%) | 4 | 55.75 | 2.36 |
| Hemoglobin (g %) | 5 | 17.78 | 1.74 |
| Plasma protein (g %) | 4 | 5.48 | 0.92 |

TABLE 6.12. MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION IN RODENTS COLLECTED NEAR COLSTRIP. April to September 1976.

| Species | Sex | MCHC[1] |
|---|---|---|
| *Peromyscus maniculatus* | M | 33.6 |
| | F | 33.4 |
| *Microtus orchrogaster* | M | 32.8 |
| | F | 32.6 |
| *Perognathus fasciatus* | M+F | 31.9 |

[1] MCHC = $\frac{100 \text{ (mean Hb concentration)}}{\text{mean Hct}}$, as computed from Tables 9, 10, 11

As in 1974-75, male deer mice showed higher hematocrit readings compared with females (Table 6.10). Males also showed higher hemoglobin values, which was not found in the 1974-75 data (Table 6.7). Plasma protein was identical in both sexes. The same values for *P. fasciatus* are tabulated in Table 6.11. Although the sample size is too small for adequate comparison, hematocrit and hemoglobin are a little larger than for either the deer mouse or the prairie vole, while plasma protein is somewhat smaller.

The mean corpuscular hemoglobin concentration of voles and mice in 1976 was the same for both sexes (Table 6.12) and was 4-5% higher in 1976 than for summer voles and mice in 1974-75.

Weights of several organs are presented in Table 6.13. In addition to their basic reference value, these data provide indications of functional state as well as annual cycle and life cycle trends and adjustments to environmental factors. For example, greater liver and kidney size in females is probably related to the increased metabolic demands imposed by reproduction. Mean liver weight in 13 lactating females was 1567.6 mg (S.D. = 277.5) and in 9 non-lactating females also collected in summer it was 1213.3 mg (S.D. = 257.2). The difference was highly significant ($t = 3.08$, $P < 0.005$). Mean kidney weight was also larger in these same lactating females, 363.3 mg *vs*. 233.1 mg ($t = 1.93$, $P < 0.05$).

The significance of sexual dimorphism in adrenal gland size is unclear, but it is a condition common to many eutherians (Christian, 1952; Chester-Jones, 1957). As in this study, McKeever (1964b) found that female *P. maniculatus* had larger adrenals than males. He showed that there was little seasonal change in gland size, indicating that the difference is independent of sex hormone levels, unlike the situation in *M. montanus* (McKeever, 1959). McKeever (1964b) also found adrenal size to be independent of population density, in agreement with Bendell (1959) but contrary to the findings of Christian (1959) on *P. leucopus*.

## POPULATION BIOLOGY

Five species of mice were captured in live traps: the deer mouse (*Peromyscus maniculatus*), prairie vole (*Microtus ochrogaster*), Wyoming pocket mouse (*Perognathus fasciatus*), northern grasshopper mouse (*Onychomys leucogaster*), and harvest mouse (*Reithrodontomys megalotis*). A few immature cottontails (*Sylvilagus auduboni*) were captured in fall or early winter. Occasionally a thirteen-lined ground squirrel (*Spermophilus tridecemlineatus*) or least chipmunk (*Eutamias minimus*), both diurnal species, were taken if traps were opened slightly before dusk.

This investigation is aimed primarily at nocturnal mice because of their abundance and diversity. Of the species captured, *P. maniculatus* formed an overwhelming portion of the catch. Members of this species were taken in every trapping session in which animals were captured. They accounted for 77.4% (721 of 931) of the individuals captured in the period and 76.4% (220 of 288) for the period April to June 1976. Furthermore, they represented 79.8% (1178 of 1476) of the total captures made at the grid sites. At the end of September 1976 this

TABLE 6.13. ORGAN WEIGHTS OF ADULT *Peromyscus maniculatus* COLLECTED NEAR COLSTRIP. July 1974 to August 1975[1]

| | N | Mean | S.D. | *t* | P |
|---|---|---|---|---|---|
| Heart | | | | | |
| Females | 28 | 159.9 | 28.5 | --- | --- |
| Males | 47 | 160.7 | 27.3 | 0.11 | >0.45 |
| Lungs | | | | | |
| Females | 15 | 267.6 | 65.9 | --- | --- |
| Males | 21 | 292.7 | 59.7 | 1.17 | >0.10 |
| Liver | | | | | |
| Females | 26 | 1427.0 | 317.6 | --- | --- |
| Males | 45 | 1190.1 | 271.8 | 3.19 | <0.005 |
| Spleen | | | | | |
| Females | 26 | 122.0 | 151.4 | --- | --- |
| Males | 46 | 84.3 | 48.7 | 1.24 | <0.10 |
| Kidneys | | | | | |
| Females | 33 | 345.6 | 51.5 | --- | --- |
| Males | 42 | 315.3 | 37.1 | 2.85 | <0.005 |
| Adrenals | | | | | |
| Females | 34 | 14.32 | 5.84 | --- | --- |
| Males | 43 | 10.47 | 4.42 | 3.21 | <0.005 |

[1] milligrams

figure was 71.8%, including recaptures of marked animals (Tables 6.14 and 6.15).

A capture summary of animals by species, sex and trapping location shows that the five species were distributed in about the same proportions throughout the study area, the Kluver East grid being an exception. At that location no R. megalotis were captured but 68.8% (11 of 16) of the O. leucogaster individuals taken in the 1974-5 study were captured there (Table 6.16).

Table 6.16 includes only individuals whose sex was determined. These data indicate that in all species except P. maniculatus (Table 6.17) the sex ratios were not statistically different. The sex ratio of P. maniculatus during the spring and summer was similar to that found in 1974-75 at the same site. However, 3-1/2 times as many male P. fasicatus were trapped as females.

During 1974-75, 59.3% (210 of 354) of P. maniculatus captured on grids were males. Recapture rate was 58.6% in favor of males (268 of 457). There was no indication of differental survival as seen in recapture tendencies (Figure 6.7) or in overwinter survival (Table 6.18). Collectively, this suggests that more males were trapped because more were present. The relatively low percentage of females suggests also that the P. maniculatus population was relatively low during the time of this study.

Trapping success is summarized in Table 6.19. Overall success never exceeded 10% in a given month. Having two traps per station undoubtedly contributed to the relatively low catch per trap; still, there is every indication that mouse populations were low. During a population high, for example, trapping success can be at least as great as 44% for P. maniculatus (Hoffman, 1955).

There was considerable seasonal variation in the relative abundance of species captured on the various grids (Figure 6.8). P. maniculatus was invariably the most abundant species, comprising from 52 to 95% of the total monthly catch (Figure 6.8). O. leucogaster and P. fasciatus are hibernators and their absence from samples during winter was expected. R. megalotis is probably a hibernator also. However, one was taken in January 1975 and none thereafter. A sharp decrease in the catch of M. ochrogaster occurred after October 1974. Prior to January 1975 voles comprised 13 to 33% of the catch (Lewis and Morton, 1975). This decrease in numbers of M. ochrogaster is unexplained but it could be part of a normal population cycle. Density-dependent cycles occur with a periodicity of about four to five years (Krebs, 1966; Batzli and Pitelka, 1970; Christian, 1971; Krebs et al., 1973) in microtine rodents. A period of unusually severe weather coincided with the onset of decreased catch of M. ochrogaster and R. megalotis. Winter temperatures were generally mild in comparison to long-term averages through most of January (Figure 6.9). From late January and through early March, however, temperatures were at the seasonal low or oscillating enough to cause a daily freeze-thaw cycle. Consequently, in four attempts at trapping in February and early March, not a single animal was captured. The presence of fresh tracks in the snow at trap entrances indicated that mice were abroad and active, but trap mechanisms were invariably frozen and inoperative. Poorly functioning traps also affected trapping success during periods of low temperature in November and December.

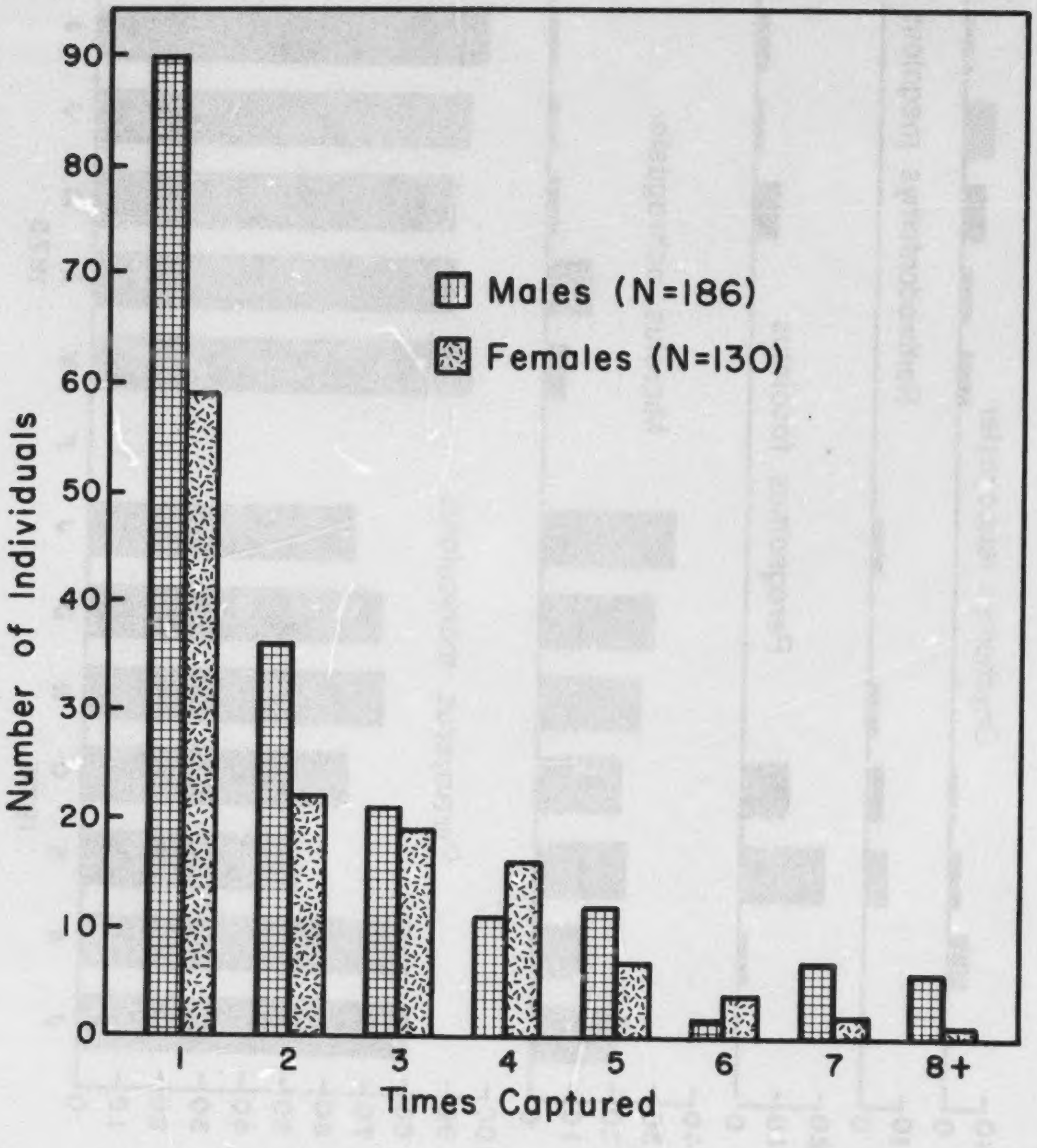

Figure 6.7. Recapture frequencies of Peromyscus maniculatus captured on grids near Colstrip, July 1975 to July 1975.

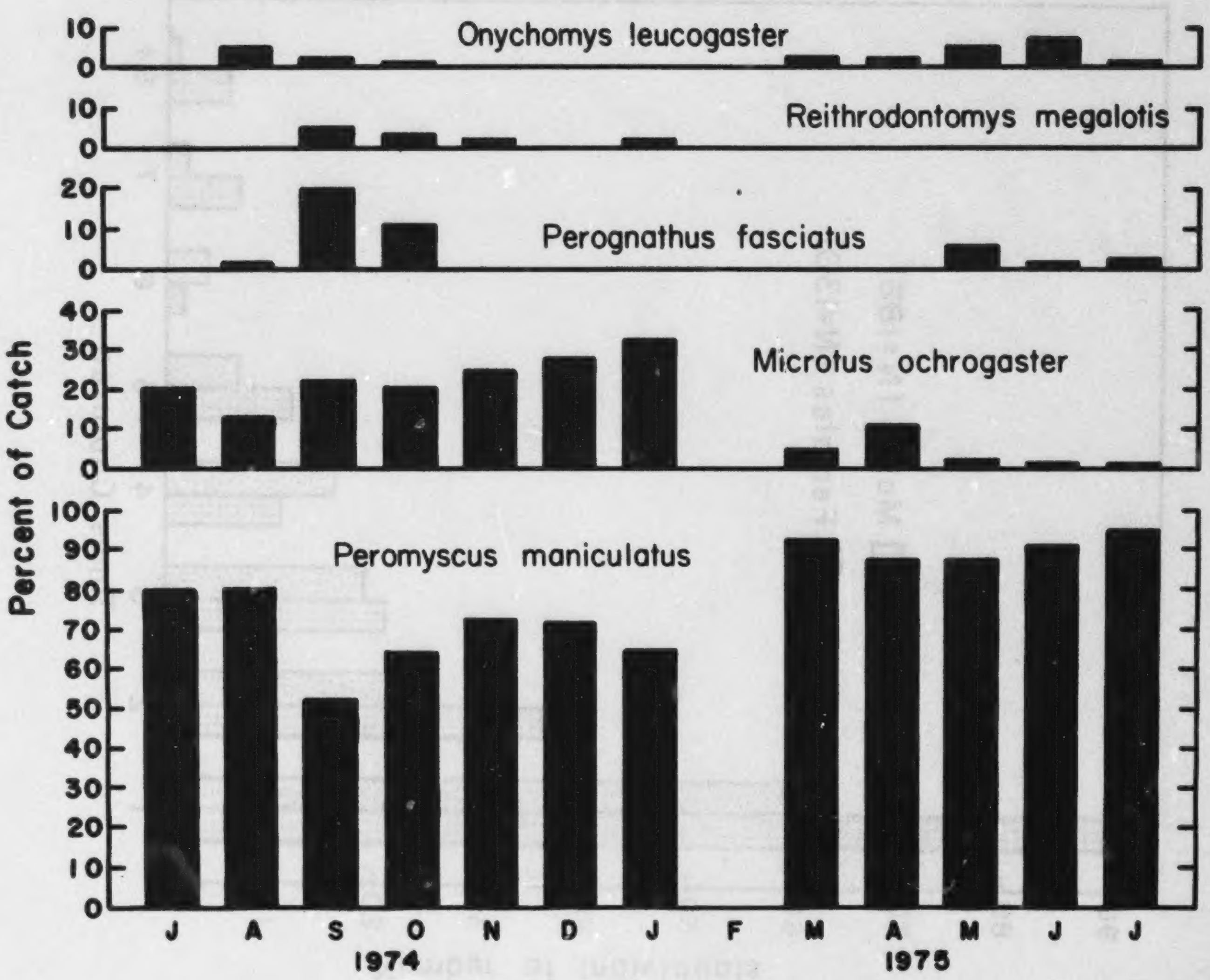

Figure 6.8. Seasonal change in relative numbers of mice captured on grids near Colstrip.

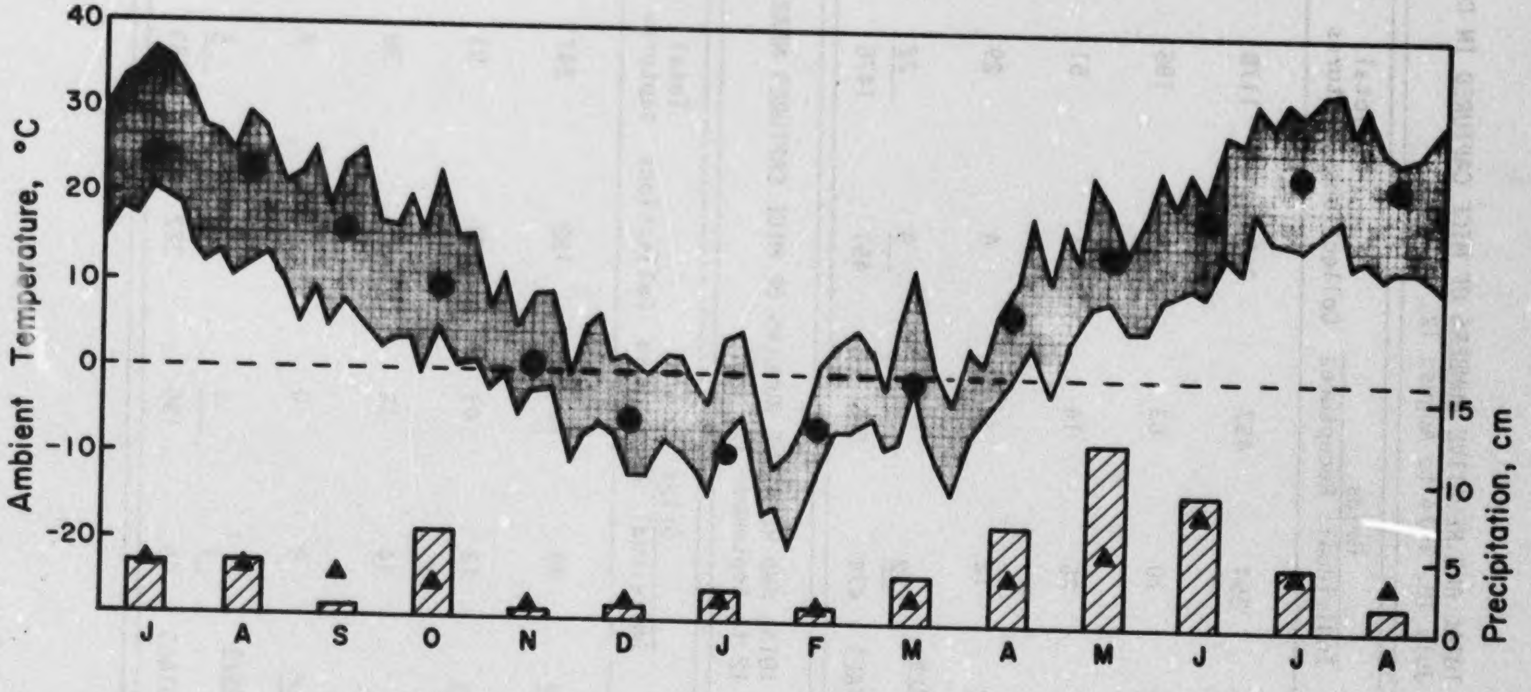

Figure 6.9. Mean daily high and low ambient temperatures summarized at 5-day intervals (shaded area) and monthly precipitation (histograms) from July 1974 through August 1975. Long-term monthly averages are shown by solid symbols. Data recorded at Miles City, Montana, airport, which is about 75 km from study sites and provided by Miles City Flight Service.

TABLE 6.14. TOTAL AND RELATIVE NUMBERS OF MICE CAPTURED IN COLSTRIP STUDY, July 10, 1974 to August 12, 1975.

| | Grids | | | | |
|---|---|---|---|---|---|
| | Individuals | Recaptures | Collections | Total captures | % of total captures |
| *Peromyscus maniculatus* | 354 | 457 | 367 | 1178 | 79.8 |
| *Microtus ochrogaster* | 70 | 63 | 63 | 196 | 13.3 |
| *Perognathus fasciatus* | 26 | 14 | 11 | 51 | 3.4 |
| *Onychomys leucogaster* | 12 | 13 | 4 | 29 | 2.0 |
| *Reithrodontomys megalotis* | 12 | 1 | 9 | 22 | 1.5 |
| TOTALS | 474 | 548 | 457 | 1476 | 100.0 |

TABLE 6.15. TOTAL AND RELATIVE NUMBERS OF MICE CAPTURED NEAR COLSTRIP, April 12 to September 10, 1976.

| | Grids | | | | |
|---|---|---|---|---|---|
| | Individual | Recaptures | Collections | Total captures | % of total captures |
| *Peromyscus maniculatus* | 38 | 127 | 182 | 347 | 71.8 |
| *Microtus ochrogaster* | 13 | 49 | 31 | 93 | 19.3 |
| *Perognathus fasciatus* | 14 | 18 | 6 | 38 | 7.9 |
| *Onychomys leucogaster* | 0 | 0 | 0 | 0 | 0 |
| *Reithrodontomys megalotis* | 1 | 1 | 3 | 5 | 1.0 |
| TOTALS | 66 | 195 | 222 | 483 | 100.0 |

TABLE 6.16. SUMMARY OF CAPTURE RECORDS OF MICE ACCORDING TO SPECIES, SEX, AND TRAPPING LOCATIONS DURING 1974-75.

| Species | McRae | | Kluver North | | Kluver East | | Kluver West | | Garfield's | | Collections |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | [1]I | [2]R | I | R | I | R | I | R | I | R | I |
| Peromyscus maniculatus | | | | | | | | | | | |
| Males | 79 | 121 | 40 | 61 | 45 | 54 | 26 | 13 | 20 | 19 | 220 |
| Females | 57 | 94 | 20 | 26 | 44 | 51 | 15 | 11 | 8 | 7 | 142 |
| Microtus ochrogaster | | | | | | | | | | | |
| Males | 20 | 18 | 4 | 3 | 5 | 4 | 1 | 0 | 9 | 5 | 33 |
| Females | 14 | 21 | 6 | 4 | 4 | 1 | 1 | 0 | 6 | 7 | 30 |
| Perognathus fasciatus | | | | | | | | | | | |
| Males | 4 | 4 | 6 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 4 |
| Females | 11 | 6 | 2 | 3 | 1 | 0 | 0 | 0 | 1 | 0 | 6 |
| Onychomys leucogaster | | | | | | | | | | | |
| Males | 0 | 0 | 0 | 0 | 5 | 6 | 0 | 0 | 0 | 0 | 3 |
| Females | 0 | 0 | 1 | 0 | 6 | 7 | 0 | 0 | 0 | 0 | 1 |
| Reithrodontomys megalotis | | | | | | | | | | | |
| Males | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 |
| Females | 3 | 1 | 4 | 0 | 0 | 0 | 1 | 0 | 2 | 0 | 3 |

[1] Number of individuals

[2] Number of recaptures

TABLE 6.17. SEX DISTRIBUTION AND SEX RATIOS OF ALL MICE CAPTURED DURING 1974-75.

| Species | Males[1] | Females[1] | Ratio | P |
|---|---|---|---|---|
| *Peromyscus maniculatus* | 430 | 286 | 1.50:1 | <0.005 |
| *Microtus ochrogaster* | 72 | 61 | 1.18:1 | NS |
| *Perognathus fasciatus* | 15 | 21 | 0.7:1 | NS |
| *Onychomys leucogaster* | 8 | 8 | 1.00:1 | NS |
| *Reithrodontomys megalotis* | 8 | 13 | 0.62:1 | NS |

[1] Numbers of recaptures not included

TABLE 6.18. OVERWINTER SURVIVAL OF *Peromyscus maniculatus*.

|  |  |  |  | Recaptured March-August 1975 |  |  |
|---|---|---|---|---|---|---|
| Age | Period of first capture | Males | Females | Males | Females | Survival % |
| *Adults* | July-August 74 | 31 | 23 | 7 | 2 | 16.7 |
|  | September-October 74 | 20 | 16 | 4 | 3 | 19.4 |
|  | November-December 74 | 9 | 3 | 2 | 0 | 16.7 |
|  | January 75 | 8 | 2 | 4 | 2 | 60.0 |
|  | TOTAL | 68 | 44 | 17 | 7 | 21.4 |
| *Immatures* | July-December 74 | 24 | 16 | 3 | 0 | 7.5 |

TABLE 6.19. SEASONAL CHANGES IN SPECIES COMPOSITION AND TRAPPING SUCCESS ON COLSTRIP GRID[1]

| Species | 1974 | | | | | | 1975 | | | | | | 1976 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | July | Aug | Sept | Oct | Nov | Dec | Jan | Mar | Apr | May | June | July | May 19 | Jun 10 | Jun 30 | Jul 21 | Aug 11 | Sep 1 |
| Peromyscus maniculatus | 51 | 130 | 95 | 76 | 33 | 36 | 31 | 28 | 98 | 76 | 77 | 80 | 30 | 33 | 29 | 18 | 25 | 30 |
| Microtus ochrogaster | 13 | 23 | 19 | 21 | 11 | 14 | 16 | 2 | 10 | 2 | 1 | 1 | 0 | 9 | 10 | 17 | 4 | 6 |
| Perognathus fasciatus | 0 | 2 | 19 | 11 | 0 | 0 | 0 | 0 | 0 | 5 | 1 | 2 | 8 | 11 | 7 | 2 | 1 | 3 |
| Onychomys leucogaster | 0 | 8 | 2 | 1 | 0 | 0 | 0 | 1 | 2 | 4 | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Reithrodontomys megalotis | 0 | 0 | 8 | 3 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| Total mice | 64 | 163 | 143 | 112 | 45 | 50 | 48 | 31 | 110 | 87 | 85 | 84 | 38 | 55 | 46 | 37 | 30 | 39 |
| Trap nights | 1452 | 3388 | 1452 | 1452 | 968 | 968 | 484 | 720 | 1210 | 1210 | 1210 | 2178 | --[2] | --[2] | --[2] | --[2] | --[2] | --[2] |
| % success, all mice | 4.4 | 4.8 | 9.8 | 7.7 | 4.6 | 5.2 | 9.9 | 4.3 | 9.1 | 7.2 | 7.0 | 3.9 | 5.2 | 8.3 | 6.6 | 5.4 | 4.2 | 5.5 |
| % success, Peromyscus only | 3.5 | 3.8 | 6.5 | 5.2 | 3.4 | 3.7 | 6.4 | 3.9 | 8.1 | 6.3 | 6.4 | 3.7 | 4.1 | 5.0 | 4.2 | 2.6 | 3.5 | 4.2 |

[1] Total numbers of animals.

[2] Approximately 720 each session.

Other investigators have reported similar difficulties (Gunderson, 1950; MacKay, 1962; Bergstedt, 1965).

Only a few mice of any species except P. maniculatus were captured during a given trapping session. In such cases there is negligible value in applying procedures or models for predicting population size; the best estimate of population size is the minimum number of animals present, that is, the number actually captured during a trapping session. This number, the minimum estimate, is shown for all grid trapping efforts (Tables 6.20 - 6.23) along with an estimate of population size for P. maniculatus by application of the method of Jolly (1965). The Jolly stochastic model was the best of four methods tested for predicting population size (French et al., 1971), but it may underestimate the population (O'Farrell et al., 1975). Only at Kluver North and McRae's were sufficient capture records obtained to predict a population substantially above that of the minimum estimate.

The similarity of Jolly's estimates to minimum estimates in many of the trapping sessions indicates that nearly all P. maniculatus present on the grids were being captured and supports the conclusion from data on trapping success and sex ratios that the population was low. The same might be inferred regarding the numbers of other rodent species, but the minimum estimates are suspect for several reasons. First, rolled oats may not be an effective bait for primarily insectivorous species such as O. leucogaster; even P. maniculatus probably prefers insects when they are readily available (Williams, 1959; Brown, 1966; Kritzman, 1974). In fact seasonal changes in acceptance of bait by rodents may influence trapping success more than population density (Fitch, 1954). Second, weather conditions were variable. The unquantifiable effect of frozen trap mechanism has been mentioned, but other factors such as rainfall can be implicated. For example, only 38 captures of P. fasciatus were made during the entire study but 14 (37%) of these occurred on one rainy night in September on the McRae grid (Table 6.20). Weather has also been identified as affecting the response of rodents to traps (Getz, 1961, 1968; Gentry et al., 1966; Weiner and Smith, 1972; Gurnell, 1976). The suitability of sage-grassland as rodent habitat is also questionable. In running collection traplines in 1975, it was noted that the tops of steep little knolls with outcrops of rocks such as those at Hay Coulee were comparatively more productive for trapping P. maniculatus, although in 1976 flat areas were also highly productive. Microtus runways were noted in numbers in old hay fields, creek bottoms, and roadside ditches but not in grid areas. The best success in catching M. ochrogaster on grids was at McRae's where a grassy swale bisected the grid and at Garfield's where a swale flanked one side and where both Pony Creek and Rosebud Creek bottoms were within a few hundred meters. Such grassy areas probably effect a patchy distribution of M. ochrogaster because of their suitability for runways rather than as food sources (Martin, 1956; Fleharty and Olson, 1969; Grant and Morris, 1971).

## HISTOLOGY

The study of histological cycles is designed to help interpret biological change at the levels previously discussed (e.g., reproduction and development, physiological condition, and population dynamics) and to aid in the identification of direct and indirect effects of pollution. Tissues under investiga-

TABLE 6.20. NUMBER OF MICE CAPTURED ON KLUVER NORTH GRID. MINIMUM ESTIMATE (ME) EQUALS TOTAL CATCH ON A GIVEN TRAPPING DATE AND JOLLY ESTIMATE (JE) WITH STANDARD ERROR EQUALS POPULATION SIZE PREDICTED BY JOLLY'S (1965) MODEL.

| Date | Peromyscus maniculatus | | | Microtus ochrogaster | Perognathus fasciatus | Onychomys leucogaster | Reithrodontomys megalotis |
|---|---|---|---|---|---|---|---|
| | ME | JE | SE | ME | ME | ME | ME |
| July 15 1974 | 5 | --- | --- | 0 | 0 | 0 | 0 |
| August 2 | 9 | 18.9 | 9.0 | 1 | 0 | 3 | 0 |
| August 13 | 9 | 16.7 | 5.4 | 0 | 0 | 0 | 0 |
| August 23 | 4 | 6.7 | 3.7 | 0 | 1 | 0 | 0 |
| September 13 | 16 | 23.1 | 6.8 | 4 | 0 | 0 | 1 |
| October 8 | 12 | 17.5 | 5.4 | 1 | 5 | 0 | 2 |
| November 5 | 11 | 21.4 | 7.8 | 1 | 0 | 0 | 1 |
| December 5 | 15 | 41.2 | 23.5 | 6 | 0 | 0 | 0 |
| March 20 1975 | 15 | 33.8 | 17.0 | 0 | 0 | 1 | 0 |
| May 6 | 14 | 16.2 | 4.4 | 1 | 1 | 0 | 0 |
| May 13 | 11 | 12.3 | 3.6 | 1 | 2 | 0 | 0 |
| May 16 | 11 | 10.0 | 3.2 | 0 | 2 | 0 | 0 |
| July 11 | 5 | 9.0 | 4.3 | 0 | 0 | 0 | 0 |
| July 15 | 3 | 12.0 | --- | 0 | 0 | 0 | 0 |
| July 18 | 3 | --- | --- | 0 | 0 | 0 | 0 |

TABLE 6.21. NUMBER OF MICE CAPTURED ON KLUVER EAST GRID. MINIMUM ESTIMATE (ME) EQUALS TOTAL CATCH ON A GIVEN TRAPPING DATE AND JOLLY ESTIMATE (JE) WITH STANDARD ERROR EQUALS POPULATION SIZE PREDICTED BY JOLLY'S (1965) MODEL.

| Date | Peromyscus maniculatus | | | Microtus ochrogaster | Perognathus fasciatus | Onychomys leucogaster | Reithrodontomys megalotis |
|---|---|---|---|---|---|---|---|
| | ME | JE | SE | ME | ME | ME | ME |
| July 23 1974 | 13 | --- | --- | 2 | 0 | 0 | 0 |
| August 6 | 8 | 9.7 | 3.3 | 1 | 0 | 3 | 0 |
| August 16 | 11 | 14.2 | 4.5 | 0 | 0 | 2 | 0 |
| August 30 | 13 | 15.3 | 4.4 | 4 | 1 | 3 | 0 |
| September 20 | 13 | 16.5 | 5.2 | 2 | 0 | 2 | 0 |
| October 12 | 8 | 8.0 | 2.8 | 1 | 0 | 1 | 0 |
| November 15 | 7 | 15.2 | 11.5 | 4 | 0 | 0 | 0 |
| December 13 | 2 | 6.0 | 5.8 | 0 | 0 | 0 | 0 |
| March 25 1975 | 8 | 8.0 | 2.8 | 0 | 0 | 0 | 0 |
| May 28 | 23 | 23.0 | 4.8 | 0 | 0 | 2 | 0 |
| May 29 | 17 | 20.5 | 4.9 | 0 | 0 | 2 | 0 |
| June 3 | 10 | 12.9 | 3.8 | 0 | 0 | 3 | 0 |
| June 6 | 11 | 24.4 | 13.4 | 0 | 0 | 3 | 0 |
| July 25 | 11 | 43.3 | 40.5 | 0 | 0 | 0 | 0 |
| July 30 | 10 | 10.0 | --- | 0 | 0 | 1 | 0 |
| August 1 | 13 | --- | --- | 0 | 0 | 0 | 0 |

TABLE 6.22. NUMBER OF MICE CAPTURED ON KLUVER WEST GRID. MINIMUM ESTIMATE (ME) EQUALS TOTAL CATCH ON A GIVEN TRAPPING DATE AND JOLLY ESTIMATE (JE) WITH STANDARD ERROR EQUALS POPULATION SIZE PREDICTED BY JOLLY'S (1965) MODEL.

| Date | Peromyscus maniculatus | | | Microtus ochrogaster | Perognathus fasciatus | Onychomys leucogaster | Reithrodontomys megalotis |
|---|---|---|---|---|---|---|---|
| | ME | JE | SE | ME | ME | ME | ME |
| July 15 1974 | 4 | --- | --- | 0 | 0 | 0 | 0 |
| August 2 | 3 | --- | --- | 0 | 0 | 0 | 0 |
| August 13 | 4 | 12.0 | 11.5 | 0 | 0 | 0 | 0 |
| August 23 | 6 | 10.5 | 5.9 | 0 | 0 | 0 | 0 |
| September 17 | 8 | 8.0 | 2.8 | 0 | 0 | 0 | 1 |
| October 11 | 12 | 12.0 | 3.5 | 0 | 1 | 0 | 0 |
| November 12 | 12 | 16.8 | 6.3 | 1 | 0 | 0 | 0 |
| December 10 | 9 | 17.1 | --- | 1 | 0 | 0 | 0 |
| May 20 1975 | 5 | --- | --- | 0 | 0 | 0 | 0 |

TABLE 6.23. NUMBER OF MICE CAPTURED ON MCRAE'S GRID. MINIMUM ESTIMATE (ME) EQUALS TOTAL CATCH ON A GIVEN TRAPPING DATE AND JOLLY ESTIMATE (JE) WITH STANDARD ERROR EQUALS POPULATION SIZE PREDICTED BY JOLLY'S (1965) MODEL.

| Date | Peromyscus maniculatus | | | Microtus ochrogaster | Perognathus fasciatus | Onychomys leucogaster | Reithrodontomys megalotis |
|---|---|---|---|---|---|---|---|
| | ME | JE | SE | ME | ME | ME | ME |
| July 10 1974 | 11 | --- | --- | 2 | 0 | 0 | 0 |
| July 28 | 14 | 20.5 | 6.4 | 6 | 0 | 0 | 0 |
| August 9 | 16 | 17.3 | 4.2 | 7 | 0 | 0 | 0 |
| August 20 | 21 | 21.0 | 4.6 | 7 | 0 | 0 | 0 |
| September 10 | 28 | 28.0 | 5.3 | 3 | 3 | 0 | 2 |
| September 27 | 18 | 27.3 | 6.7 | 7 | 14 | 0 | 2 |
| October 1 | 13 | 24.0 | 6.8 | 5 | 1 | 0 | 1 |
| October 22 | 23 | 28.7 | 7.0 | 8 | 4 | 0 | 1 |
| December 3 | 9 | 26.7 | 10.8 | 7 | 0 | 0 | 0 |
| January 25'75 | 20 | 36.2 | 10.6 | 10 | 0 | 0 | 0 |
| April 1 | 17 | 24.5 | 5.8 | 2 | 0 | 0 | 0 |
| April 4 | 21 | 28.9 | 6.2 | 4 | 0 | 0 | 0 |
| April 15 | 21 | 28.3 | 6.0 | 2 | 0 | 0 | 0 |
| April 18 | 28 | 30.4 | 6.3 | 1 | 0 | 0 | 0 |
| June 10 | 23 | 33.2 | 8.6 | 1 | 0 | 0 | 0 |
| June 17 | 16 | 22.6 | 5.3 | 0 | 1 | 0 | 0 |
| June 24 | 18 | 25.7 | 6.5 | 0 | 0 | 0 | 0 |
| July 2 | 14 | 17.9 | --- | 0 | 2 | 0 | 0 |

tion can be categorized as indicators of stress (e.g., adrenal and thyroid glands), indicators of immunological change (e.g., spleen), indicators of toxicological stress (e.g., lung, liver, and kidney), and indicators of reproductive function (e.g., gonads and accessory glands). Only the major reproductive structures of immature and adult *P. maniculatus* and *M. ochrogaster* are treated in this report. Portions of the testes and associated structures are described synoptically in Tables 6.24 to 6.31; these are further described and discussed in the associated text.

### Seminiferous tubules

The body of the testis contains many pyramidal compartments which contain one to several seminiferous tubules, each bounded by a thick tunica albuginea of regularly arranged collagen fibers. Within these tubules the spermatozoa or male germ cells are formed and are visible in the tubule walls along with developing gametes and Sertoli cells, the major supporting component of the seminiferous epithelium. A regular feature of the tubules, especially when they are spermatogenetically inactive, is one-to-few round eosinophilic droplets the size of mature primary spermatocytes. These occur in the tubular lumen or among the layers of gametes lining its wall. They have been previously described and are interpreted as degenerate gametocytes (e.g., Clarke and Kennedy, 1967).

Spermatogenic cells at various stages of development also line the tubule. These are described in order of development: spermatogonia or gonia, primary spermatocytes, secondary spermatocytes, spermatids, and spermatozoa. Spermatogonia multiply by mitosis. Frequent mitotic spermatogonia appear in the seminiferous tubules. They contain chromosomes, are small in size, and line the margins of the seminiferous tubule. However, these dividing gonia are distinct from primary or secondary spermatocytes that undergo meiosis.

### Interstitial tissue

The interstitial tissue or Cells of Leydig represent the endocrine component of the testis. They are located in areas around the convoluted seminiferous tubules. They elaborate several steroid hormones, especially testosterone, without which the seminiferous epithelium does not sustain proliferation and differentiation of the germ cells. Testosterone also supports the normal development of accessory glands of the reproductive tract, such as the seminal vesicles. When active, Leydig cells are easily recognized. When inactive, they cannot be distinguished from other connective tissue cells that lie between the seminiferous tubules. Leydig cells classified on the basis of cytoplasmic characteristics are as follows:

1. inactive: appear as other connective tissue cells, e.g., fibroblasts;

2. moderately active: round nucleus and cells, but with only a small amount of cytoplasm; nucleus occupies most of the cell volume;

3. active: round nucleus and cells; cytoplasm more abundant and frequently filled with secretory granules or small vesicles;

TABLE 6.24. GENERAL SUMMARY -- IMMATURE TESTIS *Peromyscus maniculatus*.

| | | Seminiferous Tubules | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Month | Year | Average Diameter[1] (μ ± SE) | (n) | Most Advanced Stage[2] | Mitotic Gonia | First Meiotic Figures[3] | Degenerating Gametes | Activity of Leydig Cells |
| July | 1974 | 121.5 | (1) | Spermatozoa | Many | None | None | Mod. Active |
| Aug | 1974 | 75.8 | (3) | Primaries (2)<br>Spermatozoa (1) | Some | None (1)<br>Many (2) | None (2)<br>Some (1) | Inactive (2)<br>Mod. Active (1) |
| Sept | 1974 | 73.2 ± 10.6 | (10) | Gonia (1)<br>Primaries (5)<br>Secondaries (2)<br>Spermatozoa (2) | None (1)<br>Some (2)<br>Many (7) | None (6)<br>Many (4) | None (3)<br>Some (7) | Inactive (9)<br>Mod. Active (1) |
| Oct | 1974 | 56.4 ± 4.6 | (10) | Primaries (9)<br>Spermatozoa (1) | None (1)<br>Many (9) | None (3)<br>Some (1)<br>Many (6) | None (2)<br>Some (7)<br>Many (1) | Inactive |
| Nov | 1974 | 56.3 ± 3.2 | (8) | Primaries | None (1)<br>Many (7) | None (1)<br>Some (3)<br>Many (4) | None (2)<br>Some (7)<br>Many (2) | Inactive (6)<br>Mod. Active (2) |
| Dec | 1974 | 72.0 ± 8.6 | (6) | Primaries (1)<br>Secondaries (4)<br>Spermatozoa (1) | None (4)<br>Some (2) | Some (2)<br>Many (4) | None (2)<br>Some (4) | Inactive (4)<br>Mod. Active (1)<br>Active (1) |
| Jan | 1975 | 105.6 ± 8.2 | (5) | Spermatozoa | None (2)<br>Many (3) | None (4)<br>Many (1) | None (4)<br>Some (1) | Active |
| Mar | 1975 | 152.0 | (2) | Spermatozoa | Many | None | None | Very Active |
| May | 1975 | 139.2 ± 13.9 | (4) | Spermatozoa | Some (1)<br>Many (3) | None (3)<br>Many (1) | None<br>Some (1) | Active (3)<br>Very Active (1) |
| June | 1975 | 145.0 | (3) | Spermatozoa | Many | None (1)<br>Some (1)<br>Many (1) | None | Active (2)<br>Very Active (1) |
| July | 1975 | 78.4 ± 7.6 | (14) | Primaries (2)<br>Secondaries (1)<br>Spermatids (6)<br>Spermatozoa (5) | None (8)<br>Some (3)<br>Many (2) | None (4)<br>Some (3)<br>Many (6) | None (6)<br>Some (5)<br>Many (3) | Inactive (10)<br>Mod. Active (2)<br>Active (2) |
| Aug | 1975 | 73.6 ± 18.6 | (6) | Primaries (4)<br>Spermatozoa (2) | None (2)<br>Some (2)<br>Many (2) | None (4)<br>Many (2) | None (1)<br>Some (3)<br>Many (2) | Inactive (4)<br>Mod. Active (1)<br>Very Active (1) |

[1] Microns ± standard error; number of samples in parenthesis.

[2] Primaries = primary spermatocytes; secondaries = secondary spermatocytes.

[3] Second Meiotic figures in immature testis are not included.

TABLE 6.25. GENERAL SUMMARY -- ADULT TESTIS *Peromyscus maniculatus*.[1]

| | | | | Seminiferous Tubules | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Month | Year | Average Diameter (μ ± SE) | (n) | Most Advanced Stage | Mitotic Gonia | First Meiotic Figures | Second Meiotic Figures | Degenerating Gametes | Activity of Leydig Cells |
| July | 1974 | 159.8 ± 6.1 | (4) | Spermatozoa | Many | None | None | None (3)<br>Some (1) | Active (2)<br>Very Active (2) |
| Aug | 1974 | 148.5 ± 9.6 | (5) | Spermatozoa | Many | None (4)<br>Many (1) | None | None (4)<br>Some (1) | Inactive (1)<br>Mod. Active (1)<br>Active (1)<br>Very Active (2) |
| Sept | 1974 | 133.7 ± 9.4 | (8) | Primaries (1)<br>Spermatozoa (7) | None (1)<br>Some (1)<br>Many (6) | None (1)<br>Some (1)<br>Many (6) | None | None (4)<br>Some (4) | Inactive (1)<br>Mod. Active (2)<br>Active (4)<br>Very Active (1) |
| Oct | 1974 | 90.8 ± 12.8 | (8) | Gonia (1)<br>Primaries (3)<br>Spermatozoa (4) | None (2)<br>Some (1)<br>Many (5) | None (3)<br>Some (3)<br>Many (2) | None | None (6)<br>Some (2) | Inactive (5)<br>Mod. Active (1)<br>Active (1)<br>Very Active (1) |
| Nov | 1974 | 64.0 | (1) | Primaries | Many | Many | None | ---- | Inactive |
| Dec | 1974 | 99.2 ± 8.9 | (4) | Primaries (1)<br>Spermatozoa (3) | None (1)<br>Some (3) | Many | None | None (3)<br>Some (1) | Active |
| Jan | 1975 | 102.0 | (1) | Spermatozoa | Many | Many | None | None | Active |
| Mar | 1975 | 157.0 | (3) | Spermatozoa | None (1)<br>Some (1)<br>Many (1) | Many | None | None | Active (2)<br>Very Active (1) |
| Apr | 1975 | 163.2 ± 14.0 | (7) | Spermatozoa | None (2)<br>Some (2)<br>Many (3) | None (6)<br>Some (1) | None | None | Active (1)<br>Very Active (6) |
| May | 1975 | 196.0 ± 18.0 | (10) | Spermatozoa | None (6)<br>Many (4) | None (6)<br>Some (4) | None (9)<br>Some (1) | None (9)<br>Some (1) | Active |
| June | 1975 | 162.7 ± 4.6 | (18) | Spermatozoa | Many | None (10)<br>Some (1)<br>Many (7) | None | None (17)<br>Some (1) | Mod. Active (1)<br>Active (7)<br>Very Active(10) |
| July | 1975 | 162.8 ± 5.8 | (14) | Primaries (1)<br>Spermatozoa(13) | None (3)<br>Many (11) | None (8)<br>Some (2)<br>Many (4) | None | None | Inactive (1)<br>Mod. Active (4)<br>Active (5)<br>Very Active (4) |
| Aug | 1975 | 162.9 ± 7.3 | (10) | Spermatozoa | None (2)<br>Many (8) | None (7)<br>Some (2)<br>Many (1) | None (9)<br>Many (1) | None (9)<br>Some (1) | Inactive (1)<br>Mod. Active (1)<br>Active (2)<br>Very Active (6) |

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[1] Explanations as in Table 6.24.

TABLE 6.26. GENERAL SUMMARY -- TAIL OF EPIDIDYMIS OF IMMATURE *Peromyscus maniculatus*

| Month | Year | Epithelium |  |  |  | Tubule |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|
|  |  | Type | Height |  | Cilia | Diameter |  | Contents |  |  |
|  |  |  | (μ) | (n) |  | (μ) | (n) | Sperm[4] | Empty[5] | Other[6] |
| Aug | 1974 | Col[1](1)<br>Cub[2] | 10.5 | (1) | Many | 222.5 | (1) | 1(1) | None | None |
| Mar | 1975 | Col (2)<br>Ps[3] (2) | 18.0 | (2) | Many | 113.5 | (2) | 2(2) | None | None |
| June | 1975 | Cub (1)<br>Col (3)<br>Ps (3) | 19.5 | (3) | Many | 147.0 | (3) | 3(2) | 1(0) | Fluid (1) (0) |
| July | 1975 | Cub (1)<br>Col (1) | 10.5 | (1) | Many | 226.5 | (1) | 1(1) | None | None |
| Aug | 1975 | Cub (1) | 17.8 | (2) | Some (1) | 260.5 | (2) | 2(1) | 1(0) | None |

[1] Col = simple columnar epithelium.

[2] Cub = simple cuboidal epithelium.

[3] Ps = pseudostratified columnar epithelium.

[4] 1st number indicates number of animals with some sperm. Number in parentheses are those with sperm in all tubules.

[5] 1st number indicates number of animals with some empty tubules. Number in parentheses are the number of mice with fully empty tubules.

[6] This column describes tubule contents other than sperm. The first number indicates number of animals having some of the contents described, while that in parentheses indicates those having all of the contents.

[7] Degenerating.

[8] Lumen contains granular material.

TABLE 6.27. GENERAL SUMMARY -- HEAD OF EPIDIDYMIS OF IMMATURE *Peromyscus maniculatus*[1]

| | | Epithelium | | | | Tubule | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Month | Year | Type | Height (µ ± SE) | (n) | Cilia | Diameter (µ ± SE) | (n) | Sperm[4] | Empty[5] | Contents Other[6] | | |
| Aug | 1974 | Col[1] | 15.5 | (1) | None | 50.5 | (1) | 0(0) | 1(0) | Fibroblasts | (1) | (0) |
| Sept | 1974 | Cub[2] (1)<br>Col (2)<br>Ps[3] (7) | 15.5 ± 5.6 | (9) | ? (1)<br>None (3)<br>Many (5) | 45.0 ± 7.4 | (9) | 2(1) | 8(4) | Deg.[7] lymphocytes<br>Epithelial cells or lymphocytes<br>Granules[8]<br>Fluid | (1)<br>(1)<br>(1)<br>(2) | (0) |
| Oct | 1974 | Cub (3)<br>Col (4)<br>Ps (9) | 14.1 ± 0.8 | (9) | ? (1)<br>Some (2)<br>Many (6) | 48.6 ± 6.8 | (9) | 0(0) | 9(9) | None | | |
| Nov | 1974 | Col (1)<br>Ps (3) | 14.6 | (3) | None (2)<br>Some (1) | 42.5 | (3) | 0(0) | 3(3) | None | | |
| Dec | 1974 | Col (2)<br>Ps (2) | 19.0 | (3) | None (1)<br>Many (2) | 59.0 | (3) | 0(0) | 3(2) | Granules | (1) | (0) |
| Jan | 1975 | Ps | 18.0 | (1) | Many | 71.0 | (1) | 1(0) | 1(0) | Granules<br>Deg. Sperm | (1)<br>(1) | (0) |
| Mar | 1975 | Col (2)<br>Ps (2) | 22.8 | (2) | Many | 108.0 | (2) | 2(1) | None | Sloughed epith. cells | (1) | (0) |
| May | 1975 | Cub (2)<br>Col (4)<br>Ps (2) | 20.8 ± 3.0 | (4) | Many | 108.8 ± 12.6 | (4) | 4(0) | 4(0) | None | | |
| July | 1975 | Cub (1)<br>Col (14)<br>Ps (11) | 17.8 ± 1.0 | (14) | None (2)<br>Some (1)<br>Many (11) | 78.4 ± 11.6 | (14) | 3(0) | 14(8) | Granules<br>Heterophils | (2)<br>(1) | (0) |
| Aug | 1975 | Col (3)<br>Ps (3) | 22.0 | (3) | Some (1)<br>Many (2) | 91.6 | (3) | 3(0) | 3(0) | None | | |

[1] Abbreviations and explanations as in Table 6.26.

TABLE 6.28. GENERAL SUMMARY -- TAIL OF EPIDIDMYIS OF ADULT Peromyscus maniculatus.[1]

| Month | Year | Epithelium | | | Cilia | Tubule | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Type | Height (μ ± SE) | (n) | | Diameter (μ ± SE) | (n) | Contents Sperm | Contents Empty | Contents Other |
| Sept | 1974 | Cub (1)<br>Col (5)<br>Ps (3) | 17.9 ± 0.7 | (5) | Many | 165.1 ± 12.1 | (5) | 5(5) | None | None |
| Oct | 1974 | Col | 15.8 | (2) | Many | 200.5 | (2) | 2(2) | None | None |
| Mar | 1975 | Cub (1)<br>Col (1) | 11.0 | (1) | Many | 113.5 | (1) | 1(1) | None | None |
| Apr | 1975 | Cub (5)<br>Col (6) | 12.8 ± 0.6 | (6) | Many | 219.5 ± 12.7 | (6) | 6(6) | None | None |
| May | 1975 | Cub (3)<br>Col (5) | 12.7 ± 0.7 | (5) | Many | 226.1 ± 7.0 | (5) | 5(2) | 3(0) | Fluid (1) (0) |
| June | 1975 | Cub (6)<br>Col (7)<br>Ps (1) | 13.4 ± 1.0 | (7) | Many | 261.5 ± 11.8 | (7) | 7(6) | 1(0) | None |
| July | 1975 | Cub (3)<br>Col (8)<br>Ps (4) | 20.4 ± 2.1 | (8) | Many | 170.8 ± 9.5 | (8) | 8(1) | 7(0) | None |
| Aug | 1975 | Cub (2) | 13.3 ± 1.5 | (5) | Many | 192.6 ± 18.6 | (5) | 5(4) | 1(0) | None |

[1] Abbreviations and explanations as in Table 6.26.

TABLE 6.29. GENERAL SUMMARY -- HEAD OF EPIDIDYMIS OF ADULT *Peromyscus maniculatus*.[1]

| Month | Year | Epithelium | | | Epithelium | Tubule | | Tubule | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Type | Height (μ ± SE) | (n) | Cilia | Diameter (μ ± SE) | (n) | Contents | | | | |
| | | | | | | | | Sperm | Empty | Other | | |
| July | 1974 | Col<br>Ps | 20.5 ± 1.8 | (1) | Many | 122.5 ± 3.4 | (1) | 1(0) | 1(0) | None | | |
| Sept | 1974 | Col (5)<br>Ps (6) | 22.8 ± 2.1 | (7) | Many | 80.6 ± 7.6 | (7) | 5(2) | 5(0) | Deg. sperm<br>Granules<br>Monocytes | (2)<br>(1)<br>(1) | (0) |
| Oct | 1974 | Col (2)<br>Ps (8) | 19.1 ± 1.8 | (8) | ? (1)<br>Many (7) | 66.3 ± 8.0 | (8) | 2(1) | 7(4) | Deg. sperm<br>Prim. spermatocytes<br>Pycnotic nuclei & debris<br>Fluid | (1)<br>(1)<br>(1)<br>(2) | (0) |
| Dec | 1974 | Col<br>Ps | 24.5 | (1) | Many | 77.0 | (1) | 1(0) | 1(0) | None | | |
| Mar | 1975 | Col | 23.5 | (1) | Many | 123.0 | (1) | 1(1) | None | None | | |
| Apr | 1975 | Col (6)<br>Ps (4) | 18.8 ± 3.9 | (6) | Many | 109.3 ± 10.6 | (6) | 6(2) | 4(0) | None | | |
| May | 1975 | Cub (1)<br>Col (6)<br>Ps (4) | 21.5 ± 0.3 | (6) | Many | 116.2 ± 7.5 | (6) | 6(2) | 4(0) | None | | |
| June | 1975 | Cub (1)<br>Col (18)<br>Ps (17) | 22.6 ± 1.5 | (18) | Many | 124.4 ± 6.0 | (18) | 17(7) | 19(0) | Deg. sperm<br>Monocytes<br>Fluid<br>Cell debris | (1)<br>(1)<br>(2)<br>(1) | (0) |
| July | 1975 | Col (7)<br>Ps (7) | 25.4 ± 0.8 | (7) | Many | 108.9 ± 4.0 | (7) | 7(0) | 7(0) | None | | |
| Aug | 1975 | Col (6)<br>Ps (5) | 23.9 ± 1.3 | (6) | Many | 123.8 ± 27.5 | (6) | 6(1) | 5(0) | None | | |

[1] Abbreviations and explanations as in Table 6.26.

TABLE 6.30. GENERAL SUMMARY -- HEAD OF THE EPIDIDYMIS OF ADULT *Microtus ochrogaster*[1]

| Month | Year | Epithelium |  |  | Tubule |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|
|  |  |  |  |  |  | Contents |  |  |  |
|  |  | Type | Height (μ ± SE) (n) | Cilia | Diameter (μ ± SE) (n) | Sperm | Empty | Other |  |
|  |  |  |  | Immature *Microtus* |  |  |  |  |  |
| Aug | 1974 | Ps | 13.0 (1) | Many | 47.0 (1) | None | 1(1) | None |  |
| Nov | 1974 | Col | 17.0 (1) | Many | 67.0 (1) | 1(0) | None | Fluid (1)<br>Gametes (1) | (0) |
| Dec | 1974 | Ps | 20.5 (2) | Many | 63.5 (2) | None | 2(0) | Deg sperm (1)<br>Debris (1) | (0) |
| Aug | 1975 | Col (1)<br>Ps (1) | 20.5 (2) | None (1)<br>Many (1) | 74.8 (2) | None | 2(0) | Deg sperm (1)<br>Fluid (1)<br>Monocytes (1)<br>Heterophil (1)<br>Debris | (0) |
|  |  |  |  | Adult *Microtus* |  |  |  |  |  |
| Aug | 1974 | Col (2)<br>Ps (3) | 25.3 (3) | Many | 112.5 (3) | 3(1) | 2(0) | Fluid (1) | (0) |
| Sept | 1974 | Col (1)<br>Ps (1) | 27.0 (1) | Many | 151.0 (1) | 1(1) | None | None |  |
| Mar | 1975 | Col (2)<br>Ps (2) | 26.0 (2) | Many | 95.5 (2) | 2(1) | 1(0) | None |  |
| Apr | 1975 | Cub (1)<br>Col (3)<br>Ps (3) | 18.7 (3) | Many | 85.2 (3) | 2(2) | 1(1) | None |  |
| May | 1975 | Col (2)<br>Ps (2) | 20.2 (2) | Some (1)<br>Many (1) | 107.0 (2) | 2(0) | 2(0) | None |  |
| Aug | 1975 | Ps | 30.0 (2) | Some (1)<br>Many (1) | 120.2 (2) | 2(1) | 1(0) | None |  |

[1] Abbreviations and explanations as in Table 6.26.

TABLE 6.31. GENERAL SUMMARY -- TAIL OF EPIDIDYMIS *Microtus ochrogaster*[1]

| Month | Year | Epithelium |  |  |  | Tubule |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|---|
|  |  | Type | Height (μ) | (n) | Cilia | Diameter (μ) | (n) | Contents |  |  |  |
|  |  |  |  |  |  |  |  | Sperm | Empty | Other |  |
| Immature *Microtus* |  |  |  |  |  |  |  |  |  |  |  |
| Dec | 1974 | Ps | 22.0 | (1) | Many | 206.5 | (1) | None | None | Granules (1)<br>Cell debris (1)<br>Heterophils (1) | (1) |
| Adult *Microtus* |  |  |  |  |  |  |  |  |  |  |  |
| Mar | 1975 | Col (1)<br>Ps (1) | 12.0 | (1) | Some | 244.0 | (1) | 1(1) | None | None |  |
| Apr | 1975 | Col (2)<br>Ps (2) | 24.8 | (2) | Many | 198.0 | (2) | 2(2) | None | None |  |
| May | 1975 | Col (1)<br>Ps (1) | 18.5 | (1) | Many | 298.0 | (1) | 1(0) | None | Red Droplets (1) | (0) |
| June | 1975 | Col (1)<br>Ps (1) | 18.0 | (1) | None | 263.5 | (1) | 1(1) | None | None |  |

[1] Abbreviations and explanations as in Table 6.26.

4. very active: cell round, but larger than active or moderately active cells; cytoplasm very abundant and generally filled with secretory granules or small vesicles.

### Epididymis

The epididymis is an organ comprised of a single duct formed by the fusion of many smaller excretory ducts from the testis. It stores spermatazoa, and is believed to enable the sperm to develop the ability to fertilize ova and to move normally. The epididymis is thus seen as an organ that is critical to the final stage of sperm development. The epididymis is comprised of head, body and tail. The head and tail differ markedly in histological structure. They conform to descriptions of two regions of the epididymis of field voles (Lecyk, 1962) and are thought to be equivalent to these regions.

The tubules in the head region are relatively small and round. They are lined by a simple high columnar to pseudostratified columnar epithelium reinforced by a thin capsule of connective tissue. Epithelial cells in reproductively active males have basal oval nuclei and much apical cytoplasm. The latter is filled with secretory granules and sometimes contains a single large unstained apical vacuole as well. The cells generally feature many long sterocilia. The lumen and tubule are round and regular in outline, and the tubules are separated from each other by variable amounts of vascular connective tissue. In a nonreproductive state, the tubules shrink in size, the simple epithelium diminishes in height, and the cells are densely crowded. The tubule lumen is reduced and often disappears entirely. The cilia are difficult to locate and are completely absent in many cases.

The tubules in the tail region are very large, irregular in shape, and are generally filled with sperm. The epithelium is simple low columnar to cuboidal. Cell nuclei are centrally located and the cytoplasm is scanty. Cilia are difficult to discern.

Degenerating sperm or degenerate cells resembling primary gametocytes are sometimes observed in the epididymal tubules. Occasionally, the tissue contains focal infiltrations of lymphocytes and sometimes heterophilic polymorphonuclear leucocytes in the connective tissue between the tubules.

Epithelial height and tubule diameter of adult mice in any given month exceed those of immatures in the head but not the tail of the epididymis. Furthermore, seasonal changes in epithelial height and tubule diameter are not always in the same direction. Because diameter changes in tubule lumen from month to month are larger than changes in epithelial height, diameter may be a more sensitive measure of functional changes throughout the year.

## BIRDS

### INTRODUCTION

Birds are the most visible vertebrates of the grassland ecosystem under study during the period of spring to late fall. The dominant species are the Western Meadowlark (Sturnella neglecta), Mourning Dove (Zenaidura macroura), Lark Bunting (Calamospiza melanocorys), Vesper Sparrow (Pooecetes gramineus), and Lark Sparrow (Chondestes grammacus). The Mourning Dove occurs near riparian habitats, where drinking water and the presence of trees for nesting are found, but the other species can be found in a variety of grassland or sage-grassland areas. Within these habitats, only the meadowlark seems to be well distributed. The others often show discontinuity in their distribution.

Birds are potentially important indicator species because they are relatively long-lived secondary consumers. Such forms are highly vulnerable when ecosystem damage occurs (e.g., Stickel, 1975). Furthermore, they are unlikely to show the acute effects of pollution and most likely to suddenly and belatedly exhibit severe damage when pollutants have accumulated to toxic levels in the organ-systems (Stickel, 1975). Their long lives enhance potential for accumulation of pollutants. Even low-level, non-lethal accumulations of pollutants which do not directly debilitate may have important effects on later generations of birds and mammals (summarized by Stickel, 1975 and Truhaut, 1975).

Birds are day-active and typically roost above ground, in contrast to the nocturnal fossorial mammals that are under study. The two groups will thus have very different pollutant exposure histories. Investigation of the effects of diurnal variation in power plant emissions is one of special interest and potential importance. Mourning Doves, for example, feed most intensively during the first few daylight hours and again late in the day. Clearly, this may influence both their rate of exposure and susceptibility to air pollutants. The species of birds under study employ different feeding strategies, namely, granivory (dove), insectivory and carnivory (meadowlark), or both (bunting and sparrows). If different food categories are differentially affected by pollution damage, then it should be possible to determine the effects from assay of physiological conditions. For example, sulfur dioxide reduces pollination and pollen germination and therefore the quality of seeds (Garber, 1967). Of the birds in question such an effect would be expected to affect seed-eaters selectively. Examination of these species at histological levels and integration of tissue and gross data may reveal diet-related effects of stack emissions and hence the dietary group which is most useful as a bioindicator of pollution stress.

Members of higher trophic levels, such as birds, may accumulate pollutants for long periods before overt responses are seen. As indicated in a recent Oak Ridge Workshop (Johnson, 1976), there is a general need for information concerning the sublethal and chronic, cumulative effects of pollutants. The "threshold" exposure rates that might be expected to produce various biological effects (Lewis, Glass, and Lefohn, 1975; p. 142) on organisms are not yet known, especially in areas such as Colstrip where the impacts of pollutants are continuous, but small. It is thus desirable to determine which tissues selectively accumulate pollutants (Truhaut, 1975). By identifying avian tissues that selectively accumulate or react strongly to pollutants, it may be possible to anticipate when or with how much exposure a dramatic and sudden effect will be overtly manifested in a species.

Some birds are sensitive indicators of pollution (Lewis, Glass, and Lefohn, 1975, Ch. 8). For example, pigeons living in highly industrialized areas of Japan develop lung pathologies when aerial pollution levels are only one-tenth the value of those necessary to produce analogous pathologies in human lungs. Birds appear to be particularly useful for measuring levels of air quality because of the design of their respiratory system and their broad and regular dispersion throughout the ecosystem. In contrast to bioindicating organisms of lower trophic levels, birds provide a mechanism of separately assessing the effects of aerial and dietary pollutants. The lungs and respiratory tract are particularly valuable for monitoring air pollution. In contrast, ingested pollutants probably affect the liver and perhaps the kidney selectively.

Intensive work is proceeding on the avian lung. This organism is structurally analogous to a high volume sampler involved in aerial pollutant scavenging. It is a primary target for air-borne particulates because the nasal passages have little ability to remove them from inhaled air and because flow across the respiratory surface is in only one direction (Bretz and Schmidt-Nielsen, 1971). As the studies of Japanese pigeons suggest, the avian lung is damaged by rather small amounts of toxicants from coal-fired power plants. It should be possible to determine even low emission rates and types from stacks of this kind by periodically examining the lungs of birds in impacted areas. Coal dust, silica, and the fugitive dust of agricultural activities are all somewhat different in appearance and likely different with respect to the inflammatory responses which they produce in avian respiratory tissues.

The avian lung also seems to offer unusual potential for use in pollution gradient analysis. Stack emissions settle out at distances from power sources which vary with the type of pollutant. Heavy particles settle in the immediate vicinity of the plant. However, particles dispersed in the form of aerosols are transported great distances from the source and carry toxic gases and trace metals. This, coupled with the broad and fairly regular dispersion of birds in the grassland ecosystem, should permit (1) distinguishing the effects of pollution from the effects of other environmental stresses, and (2) through pollution gradient analysis, evaluating the effects of different concentrations of stack emissions acting on birds over an extended period.

## POPULATION BIOLOGY

Census data can yield information on the relative spatiotemporal abundance of bird species. When a census is repeated at regular intervals, changes in species frequencies and dispersion patterns can be deduced and these can be related to changes in concurrently measured environmental parameters.

By application of the method described below, the investigators hope to relate changes in easily measurable descriptive parameters such as species diversity, equitability, and species richness to changes in functional relationships between species and their habitats. Of particular interest are changes attributable to the operation of the coal-fired power plants at Colstrip. This report summarizes the baseline data and suggests a format for the analysis and interpretation of future census data.

## Methods

The method employed is patterned after the North American Breeding Bird Survey (Robbins and Van Velzen, 1970). In application, the observer starts one-half hour before local sunrise and makes 60 three-minute stops at 0.5 mi (0.8 km) intervals along a predetermined route (Figure 6.10). At each stop the number of birds of each species seen in a 400 m radius and heard, regardless of distance, is recorded. Since bird species have differential individual detectability, the census data provide no information on absolute abundance (Emlen, 1971). However, it should be possible to detect changes in species relative abundance patterns by comparing data obtained during a baseline period with those obtained using the same census technique at other times.

The census route was selected to provide sampling stations at various distances from the Colstrip power plants along an anticipated gradient of pollution impact. Individual stations span a wide range of habitats including open grassland, streams, rolling hills with ponderosa pine and juniper coverage, cliffs and habitats affected by a wide range of human impacts including urban activities, railroad right-of-way, farming, ranching, and mining.

The 1975 breeding season will be considered the baseline period for present purposes since the power plants were not operational at that time. The route in Figure 6.10 was censused on nine dates during the baseline period (May 6, May 23, June 11, June 30, July 14, July 28, August 11, August 26, and September 8).

Species diversity was measured by the Shannon-Weaver function ($H' = -\sum_{i=1}^{s} p_i \log_2 p_i$) (Shannon and Weaver, 1949), species richness (S) was measured by the number of species present, and equitability (E) was measured by $H'/\log_2 S$ (Pielou, 1977). To inspect overall temporal trends in these parameters, total H', total S, and total E were calculated from lumped census data for all sites on each sampling date. Spatial and site specific trends were inspected by calculating total across season H', S, and E from lumped data from all sampling dates for each site.

## Results and Discussion

Evaluation of pollution impact depends heavily upon a comparison of future species frequencies and dispersion patterns with baseline patterns established prior to power plant operation. It is assumed that the parameters measured are not in the process of reaction to the environmental influences extrinsic to the ecosystem under scrutiny. Limited data from the U.S. IBP Grassland Biome program indicate that grassland bird species diversity, equitability, and species richness as measured in the present study do not vary substantially between years, but generally decrease along a gradient of decreasing primary production (Wiens, 1974, Table 6.3). H', E, and S are apparently buffered from response to normally encountered environmental variations but may respond to changes in productivity of the ecosystem brought about by chronic air pollution. If so, the roadside bird census may provide an inexpensive method of indexing long-term ecosystem response to chronic pollution.

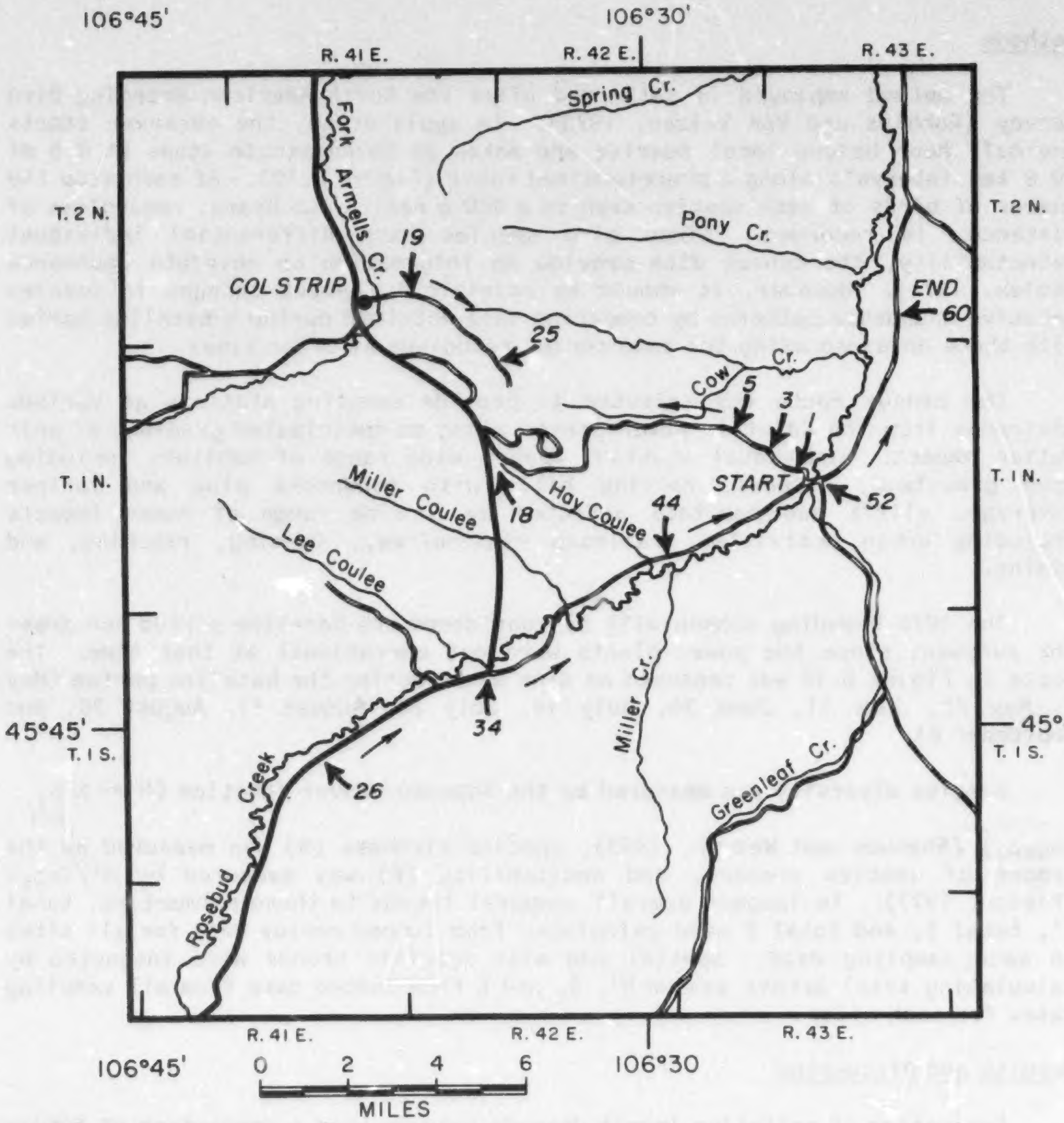
106°45'
106°30'
R. 41 E.
R. 42 E.
R. 43 E.
Spring Cr.
Fork Armells Cr.
T. 2 N.
T. 2 N.
Pony Cr.
19
COLSTRIP
END
60
25
Cow Cr.
5
3
T. 1 N.
T. 1 N.
START
52
Miller Coulee
18
Hay Coulee
44
Lee Coulee
34
45°45'
45°45'
T. 1 S.
T. 1 S.
Creek
26
Miller Cr.
Greenleaf Cr.
Rosebud
R. 41 E.
R. 42 E.
R. 43 E.
106°45'
0
2
4
6
MILES
106°30

Figure 6.10. Map of the Rosebud-Colstrip roadside census route.

Temporal trends in species frequencies are presented in Table 6.32. Though 63 species were recorded on the census route during 1975, most were relatively rare. Meadowlarks were clearly predominant and the six most common species contributed nearly 62% of total bird abundance. Diversity (H') peaks in late May, decreases until late July, and then remains fairly stable into September. Seasonal trends in E and S are not as clearcut, but the trend in H' reflects the interaction of these two components of diversity.

There is no statistically significant correlation between distance from the power plant and H', E, or S in the baseline data (Table 6.33). For all three least squares regressions, the coefficient of correlation is less than 0.7 ($p > 0.05$). Total across-season diversity ranges from 1.28 at site 5 to 3.92 at site 39. In temperate regions bird species diversity is correlated with foliage height diversity (MacArthur and MacArthur, 1961). Diversity increases with the number of layers in vegetation and with the evenness of foliage apportionment among layers. Site 5 is an open grassland, one-layered habitat. Site 39 is riparian. Grassland, shrubs and trees occur within 400 m providing a three-layered terrestrial habitat as well as an aquatic habitat for shore and water birds. It may be possible to account for much of the site variation in bird species diversity by variations in habitat diversity. An attempt is being made to quantity habitat diversity at the study sites during the 1976 field season.

Average cross season H' and S for birds in the pine savannah southeast of Colstrip are intermediate between typical values for grasslands and those for shrublands (bottom of Table 6.33). This is expected since the sampling stations range from open grassland to forest.

Average E is lower than would be predicted by the trends for H' and S. This may be an artifact of the census method which tends to overestimate meadowlark abundance relative to other species yielding generally lower E values than would otherwise be found. Variability in H', S, and E is very similar to that found in other grassland and shrubland censuses (Tramer, 1969).

H' is dependent upon both species richness and equitability. In general, bird species diversity correlates strongly with species richness while equitability tends to be high with little variability regardless of species richness. For 267 breeding bird censuses from many habitat types, Tramer (1969) demonstrated that: $H' = 0.941 (\log_2 S) - 0.251$ ($r = 0.972$). The same trend occurs in the baseline across season diversity data in the present study. The least squares regression line ($H' = 0.999 \pm 0.075$ S.E. $\times \log_2 S - 0.889 \pm 0.255$ S.E.) is similar to the general trend demonstrated by Tramer, but H' and $\log_2 S$ are not as strongly correlated ($r = 0.754$).

The components of H' (S and E) may respond differently on exposure to environmental stress. In general, high species richness and high equitability are characteristics of late successional stages (Margalef, 1963, 1968; Odum, 1969). Stressful environments generally permit less diversity to develop than favorable environments do (Sanders, 1969; Slobodkin and Sanders, 1969).

Environmental stress is primarily determined by (1) the degree of temporal predictability in environmental conditions and (2) the degree of physiological

TABLE 6.32. SPECIES FREQUENCY ALONG THE CENSUS ROUTE DURING 1975.

| | Proportional Abundance (x $10^4$) for the given date | | | | | | | | Seasonal |
|---|---|---|---|---|---|---|---|---|---|
| Common Name | 5/6 | 5/23 | 6/11 | 6/30 | 7/14 | 7/28 | 8/11 | 8/26 | 9/8 | Total |
| Mallard | 50 | | | | | | | | | 7 |
| Red-Tailed Hawk | 17 | 108 | 27 | | | 77 | | | 27 | 24 |
| Golden Eagle | 17 | | 27 | | | | | | | 5 |
| Marsh Hawk | 66 | 27 | | | | | | | | 12 |
| Prairie Falcon | | | | | 40 | | | | | 5 |
| Peregrine Falcon | | | | 20 | | | | | | 2 |
| Sparrow Hawk | 446 | 108 | 160 | | 40 | 51 | 23 | 93 | 301 | 140 |
| Sharp-Tailed Grouse | 66 | 108 | | 20 | | | | | 27 | 24 |
| Ring-Necked Pheasant | 826 | 755 | 747 | 343 | 80 | 26 | 68 | 56 | 164 | 339 |
| Killdeer | 17 | | 27 | | | 26 | | 37 | 27 | 15 |
| Common Snipe | 17 | | | | | | | | | 2 |
| Mourning Dove | 446 | 296 | 427 | 625 | 783 | 918 | 1847 | 935 | 902 | 787 |
| Long-Eared Owl | 17 | | | | | | | | | 2 |
| Poor-Will | | | | | | 26 | | | | 2 |
| Common Nighthawk | | | | | | 102 | | | | 10 |
| Chimney Swift | | | | 242 | | | | | | 29 |
| Belted Kingfisher | | | | | 20 | | | | | 2 |
| Red-Shafted Flicker | 66 | 81 | 53 | 121 | 100 | 26 | 68 | 56 | 301 | 92 |
| Red-Headed Woodpecker | | | | | | | | | 82 | 7 |
| Eastern Kingbird | | 243 | 107 | 81 | 120 | 204 | 225 | 150 | 55 | 123 |
| Western Kingbird | | 189 | 347 | 81 | 141 | 77 | 90 | 224 | 109 | 131 |
| Cassin's Kingbird | | | 53 | | 20 | | | 19 | | 10 |
| Say's Phoebe | 50 | 27 | | | | 26 | | | 109 | 22 |
| Horned Lark | | | 27 | | | | | | | 2 |
| Barn Swallow | | 243 | 187 | 161 | 80 | 153 | 113 | 93 | | 107 |
| Cliff Swallow | | 81 | 293 | 444 | 482 | 332 | 473 | | | 228 |
| Black-Billed Magpie | 33 | 27 | 80 | | | | 23 | | | 17 |
| Common Raven | | | | | 40 | | | | | 5 |
| Common Crow | 132 | 135 | 133 | 60 | 40 | 102 | | 93 | | 77 |
| Pinon Jay | | | | 323 | | | 180 | 1458 | 27 | 249 |
| Black-Capped Chickadee | 413 | 108 | | | | 26 | 90 | 56 | 273 | 114 |
| White-Breasted Nuthatch | | | | | | | 23 | | | 2 |
| House Wren | | | | | 20 | | 23 | | | 5 |
| Winter Wren | | | | | | | 23 | | 55 | 7 |
| Brown Thrasher | | | 27 | 40 | 20 | | | | | 10 |
| Robin | 33 | 243 | 107 | 60 | 221 | 51 | | 37 | 27 | 196 |
| Wood Thrush | | | | | | | | 37 | | 5 |
| Mountain Bluebird | | 54 | 240 | 20 | 20 | 77 | | | | 39 |
| Cedar Waxwing | | | | | | | 45 | | | 5 |
| Loggerhead Shrike | 50 | | 27 | | 20 | | 45 | | | 17 |
| Starling | 132 | | | | | | | | | 19 |
| Yellow Warbler | | 162 | 53 | 60 | 80 | 51 | 90 | 19 | 27 | 56 |

(continued)

TABLE 6.32. (continued )

| Common Name | Proportional Abundance (x $10^4$) for the given date<br>5/6 | 5/23 | 6/11 | 6/30 | 7/14 | 7/28 | 8/11 | 8/26 | 9/8 | Seasonal<br>Total |
|---|---|---|---|---|---|---|---|---|---|---|
| Yellow Throated Warbler | 17 | | 27 | | | 26 | | 19 | | 10 |
| Ovenbird | | | | | | | 23 | | | 2 |
| Yellow-Breasted Chat | | 27 | | 60 | 20 | | | 37 | | 17 |
| American Redstart | | | 27 | 20 | | | | | | 5 |
| Western Meadowlark | 4645 | 3396 | 3627 | 3347 | 3775 | 3163 | 3176 | 2991 | 2787 | 3448 |
| Red-Winged Blackbird | 413 | 593 | 640 | 444 | 482 | 77 | 90 | 467 | | 361 |
| Bullock's Oriole | | | 27 | | | | | | | 2 |
| Brewer's Blackbird | 1058 | 350 | 243 | 383 | 1104 | 2168 | | | 137 | 605 |
| Common Grackle | | | 53 | 20 | 20 | | | | 27 | 12 |
| Brown-Headed Cowbird | | | 107 | 262 | 261 | 102 | 203 | 280 | | 140 |
| Lazuli Bunting | | | | | 80 | 26 | 45 | 37 | | 22 |
| American Goldfinch | | | | 81 | 20 | 179 | 90 | 75 | 82 | 56 |
| Red Crossbill | | | | | | | | | 410 | 36 |
| Rufous-Sided Towhee | 33 | 108 | | | 60 | | 68 | 56 | | 36 |
| Lark Bunting | | 755 | 987 | 1815 | 863 | 510 | 1779 | 2056 | 2022 | 1165 |
| Savannah Sparrow | 331 | 189 | 160 | 60 | 40 | 77 | 135 | 75 | 219 | 157 |
| Vesper Sparrow | 430 | 539 | 187 | 343 | 321 | 306 | 405 | 393 | 1667 | 479 |
| Lark Sparrow | 132 | 943 | 747 | 444 | 522 | 995 | 541 | 93 | 137 | 465 |
| Chipping Sparrow | | 108 | 27 | 20 | 60 | 26 | | 56 | | 31 |
| Clay-Colored Sparrow | | | | | | | | | 55 | 5 |
| White-Crowned Sparrow | 33 | | | | | | | | | 5 |
| N(x$10^0$) | 605 | 371 | 375 | 496 | 498 | 392 | 444 | 535 | 368 | |
| H' | 3.14 | 3.66 | 3.59 | 3.47 | 3.40 | 3.29 | 3.22 | 3.23 | 3.29 | 3.77 |
| E | 0.65 | 0.76 | 0.71 | 0.71 | 0.67 | 0.67 | 0.67 | 0.67 | 0.70 | 0.63 |
| S | 28 | 28 | 33 | 29 | 33 | 30 | 28 | 28 | 26 | 63 |

TABLE 6.33. SPECIES DIVERSITY, EQUITABILITY, AND SPECIES RICHNESS AT THE SAMPLING STATIONS DURING 1975

| Site number | Distance from Power Plant (km) | Species Diversity (H') | Equitability (E) | Species Richness (S) |
|---|---|---|---|---|
| 1 | 15.8 | 2.70 | .73 | 13 |
| 2 | 15.0 | 1.35 | .58 | 5 |
| 3 | 14.2 | 2.47 | .65 | 14 |
| 4 | 13.6 | 2.36 | .66 | 12 |
| 5 | 12.8 | 1.28 | .55 | 5 |
| 6 | 12.0 | 1.56 | .60 | 6 |
| 7 | 11.4 | 2.74 | .70 | 15 |
| 8 | 10.6 | 2.97 | .78 | 14 |
| 9 | 9.9 | 2.89 | .78 | 14 |
| 10 | 9.3 | 3.47 | .85 | 17 |
| 11 | 8.6 | 3.29 | .77 | 19 |
| 12 | 9.0 | 2.83 | .79 | 12 |
| 13 | 9.1 | 1.84 | .58 | 9 |
| 14 | 8.5 | 1.82 | .71 | 6 |
| 15 | 8.2 | 1.61 | .57 | 7 |
| 16 | 7.5 | 2.44 | .81 | 8 |
| 17 | 6.7 | 2.24 | .80 | 7 |
| 18 | 5.9 | 1.69 | .66 | 6 |
| 19 | 1.3 | 1.89 | .60 | 9 |
| 20 | 1.9 | 1.31 | .46 | 7 |
| 21 | 2.7 | 2.20 | .64 | 11 |
| 22 | 3.5 | 2.40 | .72 | 10 |
| 23 | 4.5 | 2.69 | .85 | 9 |
| 24 | 5.1 | 2.03 | .72 | 7 |
| 25 | 5.8 | 1.69 | .84 | 4 |
| 26 | 16.2 | 1.67 | .60 | 7 |
| 27 | 15.7 | 3.38 | .87 | 15 |
| 28 | 15.2 | 2.97 | .78 | 14 |
| 29 | 14.7 | 2.88 | .76 | 14 |
| 30 | 14.4 | 1.82 | .61 | 8 |
| 31 | 14.1 | 2.03 | .61 | 10 |
| 32 | 14.1 | 3.05 | .78 | 15 |
| 33 | 14.1 | 3.29 | .82 | 16 |
| 34 | 14.1 | 2.95 | .70 | 19 |
| 35 | 13.9 | 2.62 | .87 | 8 |
| 36 | 13.8 | 1.64 | .71 | 5 |
| 37 | 13.3 | 2.50 | .75 | 10 |
| 38 | 13.0 | 3.33 | .80 | 18 |
| 39 | 13.0 | 3.92 | .89 | 21 |
| 40 | 13.0 | 2.11 | .64 | 10 |
| 41 | 13.0 | 1.95 | .76 | 6 |
| 42 | 13.1 | 1.63 | .52 | 9 |
| 43 | 13.4 | 2.70 | .75 | 12 |

(continued)

TABLE 6.33. (continued)

| Site number | Distance from Power Plant (km) | Species Diversity (H') | Equitability (E) | Species Richness (S) |
|---|---|---|---|---|
| 44 | 13.9 | 3.09 | .89 | 11 |
| 45 | 14.2 | 2.70 | .78 | 11 |
| 46 | 14.6 | 2.78 | .78 | 12 |
| 47 | 14.9 | 2.26 | .65 | 11 |
| 48 | 15.2 | 2.32 | .67 | 11 |
| 49 | 15.7 | 2.45 | .77 | 9 |
| 50 | 15.8 | 2.40 | .69 | 11 |
| 51 | 16.2 | 2.47 | .69 | 12 |
| 52 | 16.8 | 3.56 | .94 | 14 |
| 53 | 17.3 | 2.84 | .86 | 10 |
| 54 | 17.4 | 2.91 | .81 | 12 |
| 55 | 17.6 | 3.03 | .84 | 12 |
| 56 | 17.8 | 2.80 | .78 | 12 |
| 57 | 17.9 | 2.48 | .72 | 11 |
| 58 | 18.2 | 2.62 | .76 | 11 |
| 59 | 18.2 | 2.95 | .80 | 13 |
| 60 | 18.2 | 2.22 | .64 | 11 |
| Average ± 2 Standard Errors | | 2.47 ± 0.16 | 0.73 ± 0.02 | 10.95 ± 0.98 |
| Typical Grassland Values[1] | | 1.93 ± 0.24 | 0.84 ± 0.034 | 5.74 ± 1.00 |
| Typical Shrubland Values[1] | | 3.14 ± 0.16 | 0.85 ± 0.024 | 14.08 ± 2.31 |

[1] from Tramer (1969)

stress imposed (Slobodkin and Sanders, 1969). Either a temporally unpredictable or a temporally constant but physiologically stressful environment should constrain the development of high species richness since relatively few species would be able to successfully colonize the environment. However, equitability in the distribution of individuals should be different in the two environments. In a constant but severe environment, equitability should be relatively high in late successional stages. As the species which have successfully adapted to the extreme environment begin to realize full exploitation of their available resources, competition may become the dominant selective pressure and this should lead to greater equitability (Emlen, 1973). In an unpredictable environment, however, catastrophic disturbances may keep the ecosystem at a low state of maturity and should lead to a log-normal distribution of species abundances (MacArthur, 1960). As a result, both species richness and equitability would be low.

Figure 6.11 illustrates the combinations of cumulative S and E values which occurred at the 60 sampling stations during 1975. The quadrants represent the trends in species discrimination of their environments at climax expected from the previous discussion. This format may prove useful in interpreting shifts in positions of the plotted points that occur in the future. Vectors drawn from the baseline point positions for a particular site to its new position at some future time can be used to quantify the reaction of the avian community at that site to changes in environmental conditions. The vector sum for all 60 stations can be used to quantify the new shift away from baseline of the avian community along the census route and may provide a useful index of initial response to operation of the Colstrip power plants and permit tracking of subsequent ecological succession.

Table 6.34 illustrates the interpretation that can be given to various temporal shifts in vector direction. Vector length can be used to quantify the magnitude of the vector shift. This technique permits evaluation only of quantitative shifts in species numbers and relative abundances. Qualitative changes in species composition may be of equal or greater importance. Comparisons of the life history strategies of those species favored by pollution with those less favored may lead to better understanding of more general effects manifested by the ecosystem of interest. Thus where generalist species tend to be replacing or increasing at the expense of specialists, retrograde succession may be indicated.

## HISTOLOGY

### Avian Tissues As Indicators of Pollution Challenge

Histological evaluation of selective tissues and organs of birds is in progress. Baseline definition will be complete in about a year. Only those tissues that are likely to respond to the low levels of emissions expected from the Colstrip plants are examined histologically. These include the following:

Tissues that are indicators of "stress." The responses of mammals to stress are well documented (e.g., see Christian, Lloyd, and Davis, 1965) and generally fit the "general adaptation syndrome" originally promulgated by Hans Selye (von Faber, 1964). Early responses occur in the adrenal gland, particularly in the

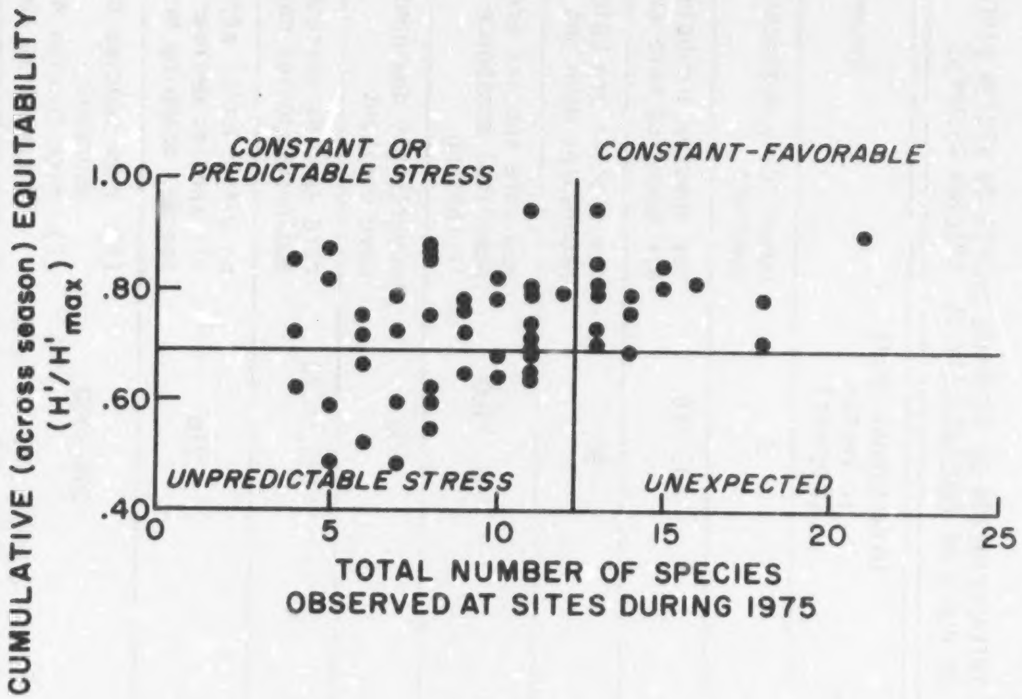

Figure 6.11. Species richness versus cumulative equitability for the 60 sampling stations during the baseline period (1975).

TABLE 6.34. INTERPRETATION OF TEMPORAL SHIFTS IN VECTOR DIRECTIONS ON PLOTS OF EQUITABILITY VS. SPECIES RICHNESS

| Δ Species Richness (S) | Δ Equitability (E) | Directional Shift in Vector (Degrees) | Interpretation |
|---|---|---|---|
| o | + | 0 | numerically dominant species becoming less dominant |
| + | + | >0, <90 | new species are being added, but all species are becoming more even numerically |
| + | o | 90 | new species are being added but relative abundances have not changed |
| + | - | >90, <180 | new rare species are being added numerical dominance by abundant species is increasing |
| o | - | 180 | numerically dominant species are becoming more dominant |
| - | - | >180, <270 | rare species decreasing and/or dropping out, dominant species may be increasing |
| - | o | 270 | no differential effect on relative abundance all species decreasing numerically, rare species dropping out |
| - | + | >270,<360 | (1) rare species drop out, others remain abundant<br>(2) rare species drop out, dominant species become less abundant |

adrenal cortex; this tissue hypertrophies and produces unusually large amounts of glucocorticoids. Lymphatic organs involute secondarily and predictable changes also take place in the blood (including lymphocytopenia and neutrophilia). These functional changes are accompanied by changes in tissue structure.

1. Adrenal Gland—There are surprisingly few studies of stress-related changes in the adrenal glands of birds, but those available are in harmony with the above picture of stress on the mammalian adrenal gland. Stressors such as oil, kepone, and high population density are known to immediately increase the weight of the gland (Flickinger, 1961; Gorman and Milne, 1971; Eroschenko, 1973). However, the adrenal gland of the bird is a mixture of interrenal and chromaffin tissue and changes in weight indicate little about which component is affected. In fact, changes in the weight of the gland are sometimes misleading. Seasonal changes in the adrenal gland of the Wood Pigeon provide an instructive example (Ljunggren, 1969). To begin with, there are no marked seasonal changes in the weight of the gland. Yet, on a histological level, there are rather pronounced seasonal variations in the activity of interrenal and chromaffin tissue. Furthermore, the male adrenal is generally heavier than that of the female. On a weight basis, it might be concluded that males are stressed more than females. However, just the opposite appears to be true: the fractional volume of cortical tissue in the female's gland always exceeds that in the male's gland.

Among the few histological studies that exist concerning birds, stress-related changes in the adrenal include (1) hypertrophy and hyperplasia of interrenal and chromaffin cells, (2) changes in the fractional volumes of interrenal and chromaffin tissue, (3) increases in the number and size of nuclei within interrenal cells, and (4) increases in the vascularity of the gland (Stoewsand and Scott, 1964; Ljunggren, 1969; Gorman and Milne, 1971; Bhattacharyya and Ghosh, 1972; Bhattacharyya, Ray, and Manna, 1972; Eroschenko, 1973). The subject is well reviewed by von Faber (1964).

Few seasonal studies of the adrenal gland exist and the functional significance of seasonal changes in this gland are not well established. In all species examined to date except the Brant, adrenal activity is reduced during periods of molt, but elevated at breeding (summarized in Gorman and Milne, 1971). Changes in the adrenal weight of Western Meadowlarks and Mourning Doves during 1975 at Colstrip (Lewis, unpublished) are consistent with these earlier studies— adrenal weight increased during the breeding season and diminished during the postnuptial molt.

2. Thyroid Gland—Manifestations of stress at the level of thyroid gland include losses of colloid and increases in the height of the follicular epithelium. Both changes are indicative of elevated glandular activity. Little, if any, concurrent change takes place in the weight of the gland (Ljunggren, 1968) which underscores the need for its histological evaluation.

The adrenal and thyroid glands at the tissue level are potentially sensitive and quick-responding bioindicators of (1) the normal stressors in the Colstrip ecosystem and (2) the additional stress of power plant emissions. Blood levels of corticosterone in key avian species will be measured in future summers at the site. It will be interesting to compare changes in the histology of the interrenal tissue and alterations in corticosterone production.

Tissues that belong to the immune system. Tissues in this category react directly to foreign materials in the body or are secondarily affected by the induced activity of the adrenal gland.

1. General—The immune system of the bird includes lymphoid cells that originate in the thymus (T lymphocytes), bone marrow (BM lymphocytes), and bursa of Fabricius (B lymphocytes). These cells disseminate to lymphatic tissue throughout the body and are responsible for immune responses. Descendants of T cells produce substances which destroy foreign bodies, such as skin grafts, outright. Descendants of BM and B cells produce nonspecific and specific antibodies, respectively. There is accumulating evidence that T cells are also involved in activities traditionally ascribed to BM and B cells. The entire subject of avian immunity was reviewed recently by Moticka (1975).

2. Bursa of Fabricius—The bursa is a lymphoepithelial gland which develops as an outgrowth of the proctodaeum. It is necessary for the development of antibody-mediated responses. It normally involutes when a bird reaches sexual maturity. However, it will involute earlier if the younger bird is subjected to stress (von Faber, 1964). It will, for example, regress in the presence of glucocorticoids (Glick, 1964; Siegel, 1971). Consequently it should be very useful for identifying stressors that affect young birds, as opposed to those which affect the avian population as a whole. Sudden regressive changes in the structure of the bursa are more likely to be translated quickly into histological changes than into reductions in the weight of the gland. This underlines the importance of examining the tissue structure of the gland.

3. Thymus—The thymus is a multilobed gland which is distributed along the jugular veins in the neck. It is diffusely arranged and difficult to remove totally, though we are generally able to do this successfully. The thymus of many avian species, including the Mourning Doves and Lark Buntings collected at Colstrip (Lewis, unpublished), enlarges during molt (Höhn, 1956; Anderson, 1970; Kendall, 1975; Ward and Kendall, 1975). Periodic rejuvenation of the gland at this time appears to provide erthrocytes to meet the demands of molt and perhaps additional lymphocytes to deal with stresses additional to that of the molt itself (Ward and D'Cruz, 1968; Ward and Kendall, 1975). The thymus involutes if the adrenal gland becomes hyperactive. Immediate and predictable thymic responses include lymphopenia, eosinopenia, and heterophilia. Consequently, it may be particularly sensitive to the stress of chronic, low-level pollution challenge. Stress-related effects are more likely to be registered first as alterations in tissue structure and only later as changes in organ weight.

4. Spleen—The spleen has been largely ignored by avian biologists (Ewart and McMillan, 1970). Since it is not a storage organ for blood, changes in its weight may be true reflections of different states of its activity. In this respect, some literature concerning seasonal changes in its weight is available for reference (Riddle, 1928; Oakeson, 1953). Its major functions are haemopoiesis, antibody production, and filtration of the blood (as part of the

mononuclear phagocyte system). Splenic leucopoiesis is influenced by the activity of the adrenal gland and by antigen challenges. Foreign proteins and carbohydrates, for example, stimulate its production of plasma cells and antibodies (Jankovic and Isakovic, 1966). The phagocytes of its red pulp also selectively engulf blood-borne particulate matter. The number and activity of the germinal centers and the activity of the follicular tissue in its white pump are directly related to its antibody production. In addition, the activity of parafollicular areas of its white pulp varies directly with the number of cell-mediated immune responses taking place within the birds. In other words, splenic tissue provides information about the magnitude of immune responses and also permits one to further determine if the responses are primarily humoral, cell-mediated, or both.

The value of the spleen as an early indicator of pollution challenge is suggested by the studies of Zarkower (1972) with mice. He demonstrated that air containing carbon, sulfur dioxide, or combinations of the two, suppresses immune responses. These pollutants destroy cells of the immune system selectively (Piliero, 1970) and their effects should be expressed relatively early in the structure of lymphoid tissues.

Detoxifying tissues. The major site of detoxification in the vertebrate body are the liver, kidney, and lungs.

1. Liver—The liver has a large number of functions, only one of which is the detoxification of endogenous and foreign substances which pass through it. These other functions probably reduce its utility as a specific indicator of pollution challenge. This is illustrated by the fact that liver weight varies with age, diet, season, stage of the reproductive cycle, and even time of the day (Farner, 1960; Hartman and Brownell, 1961; Ljunggren, 1968). Weight variations such as these underscore the importance of examining hepatic tissue as a possible indicator of pollution effects, rather than relying on changes in its weight which are relatively insensitive at best.

Changes in hepatic structure of birds in the Colstrip area in response to chronic exposure to trace metals and other pollutants may occur. Toxicants such as ethanol and carbon tetrachloride produce cirrhosis; and kepone causes focal necrosis and marked congestion in the liver (Huber, 1965). Trace metals, such as arsenic and copper, that occur in stack emissions, produce fatty degeneration and necrosis of hepatic parenchyma (Smith and Jones, 1961). Gases such as CO, also found in stack emissions, directly suppress the detoxifying functions of rat liver (Cooper et al., 1965; Conney et al., 1968), whereas chlorinated hydrocarbons such as DDT enhance the catabolic functions of avian liver (Peakall 1970). In addition to direct pollution-related alterations of hepatic parenchyma, the liver's population of Kupffer cells (as part of the mononuclear phagocyte system) may accumulate foreign particulate material at rates that correspond to environmental pollution levels.

In summary, the possibility that the liver will exhibit histological changes in birds from chronically impacted areas is very real and needs examination. In addition, the liver may be useful for distinguishing between the effects of ingested pollutants and those acquired via the respiratory system.

2. Kidney—The kidney is the second major site of detoxification in the vertebrate organism as well as the major site of excretion. It is directly damaged by pollutants such as copper, fluorine, lead, and mercury, all of which occur in stack emissions (Smith and Jones, 1961). Damage to the kidney's filtration apparatus is indicated by the presence of protein (casts) within renal tubules. There may be complete loss of renal tubules, or simply disintegration of their epithelia. Congestion, the accumulation of fluid between tubules, and the presence of fibrin in the renal corpuscule are other common pathologies. Reliable and thorough histological descriptions of the avian kidney are now available for reference (Johnson and Mugaas, 1970 a,b).

3. Lung—The respiratory tract is a third major site of detoxification in the vertebrate organism. In addition, its alveolar epithelium contains phagocytes which remove particulate matter from inhaled air. The lung is subject to direct irritation from inhaled substances; and because of its unusually large blood supply, it reacts to these substances immediately with tissue proliferation, pneumonia, focal accumulations of leucocytes, congestion, and visible damage to the alveolar wall (Smith and Jones, 1961). The lung's unusual sensitivity to air-borne pollutants is suggested by Lewis, Morton, and Jones (1975). The histology of the tissue should consequently be a particularly sensitive and selective bioindicator of air quality.

Reproductive tissues. The reproductive system is one of the first to be affected deleteriously by chronic stress (Christian and Davis, 1964; von Faber, 1964; van Tienhoven, 1968). Because this system, particularly the histological events of spermatogenesis, has been well studied in a large number of birds (Lofts and Murton, 1973), any deviations from normal in the Colstrip population should be readily appreciated.

The importance of evaluating reproductive tissues is twofold. In the short term, they should be relatively sensitive, although secondary, indicators of the stress associated with pollution impact on the ecosystem. In the long-term ecological context, they are equally important predictors of population growth in chronically polluted areas, since upsets in the reproductive function of one generation directly influence the size of the following generation.

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## SECTION 7

# REMOTE SENSING OF THE BIOENVIRONMENTAL EFFECTS OF STACK EMISSIONS IN THE COLSTRIP VICINITY

by

J. E. Taylor and W. C. Leininger

## INTRODUCTION

Procedures for ground and aerial photography have been developed to monitor air pollution effects in a grassland ecosystem. Photography offers several strong advantages for biological monitoring. Permanent, complete, periodic records can be obtained, stored, exactly reproduced, and compared over time. In this way trends and changes can be recognized and documented. Also, the relatively unselective nature of photography permits re-examination of past imagery for previously unnoticed features which later studies show to be important.

The photographic studies described here include groundlevel and relatively low elevation aerial imagery. Periodic flights allow aerial assessment of rates of vegetation changes. Ground studies help discriminate between normal and stress-induced changes. Pollution stress is detectable, but not yet quantifiable, in low-level aerial photography.

## GROUND LEVEL PHOTOGRAPHY

Ground level photography provides a detailed record of plant species, phenology, and pathologic signs. All of this contributes to the total data set and assists in the interpretation of aerial imagery. Also, vertical ground photo plots may be measured and analyzed for cover, number, frequency, pattern, and plant volume.

### PROCEDURES

Photo plots are established on all study sites and on representative examples of the various communities present in the area. Permanent meter-square plots are photographed stereoscopically in color and black-and-white. Details are given by Taylor et al., 1976.

Aspect photographs are made from vantage points within and overlooking plot areas. These are taken with color and infra-red color, the latter to compare with aerial coverage.

Ground photography is collected periodically through the growing season so that any changes will be recorded.

## DISCUSSION

A sample stereoscopic pair of photo plots appears as Figure 7.1. Species identification, canopy cover, litter, bare ground, and plant heights are among the kinds of data which can be derived from these photographs.

Plant volume, which can be used to estimate biomass, may be obtained by combining canopy coverage and height, the latter measured with a parallax wedge. Plant density and pattern also can be studied from these pictures.

For each photo plot an index of species identification has been prepared. An example is shown as Figure 7.2. Species which are diffused throughout the quadrat are termed "matrix species," and are listed but not precisely located on the indices. Species which are readily distinguishable in the plot-photographs are mapped. The combination of plot photographs and plot indices makes a permanent record of species presence and distribution. Sequential records allow the evaluation of temporal changes.

## AERIAL PHOTOGRAPHY

Low level aerial photography gives a more generalized view of plant species and community distributions, pathology, and cover than does ground photography. However, it yields more detail than higher level imagery and so represents a useful compromise between detail and comprehensiveness of coverage.

Low level aerial imagery is most practical for making detailed vegetation maps, sensing population-level stresses, or any other purposes requiring large scale synoptic views. It also aids in developing interpretations of smaller scale, high level photography.

## PROCEDURES

In this project, a Cessna 182 airplane which can easily handle the elevation range of 500 to 7000 feet above ground, was used. The plane, leased from Miles City Flying Service, Miles City, Montana, has been modified by the addition of a 12" diameter belly hole, which accepts a special mount, designed and manufactured by W.E. Woodcock of Miles City (Woodcock, 1976). The mount supports a Hasselblad EL/M motor-driven camera (70 mm format) and a manually-operated Leica MDa (35 mm). Both are fitted with 50 mm lenses, yielding negative scales from 1:3000 to 1:40,000. Other focal lengths are sometimes used to obtain changed scales without changing flight elevation. For special purposes, 80, 150, 250, and 500 mm lenses are used.

In addition to the mounted camera, oblique photographs from the air are taken with a second Hasselblad used as a hand camera. This kind of photography supplements the more traditional vertical imagery, since it is more representative of familiar aspects of scenes for interpretation, display, etc.

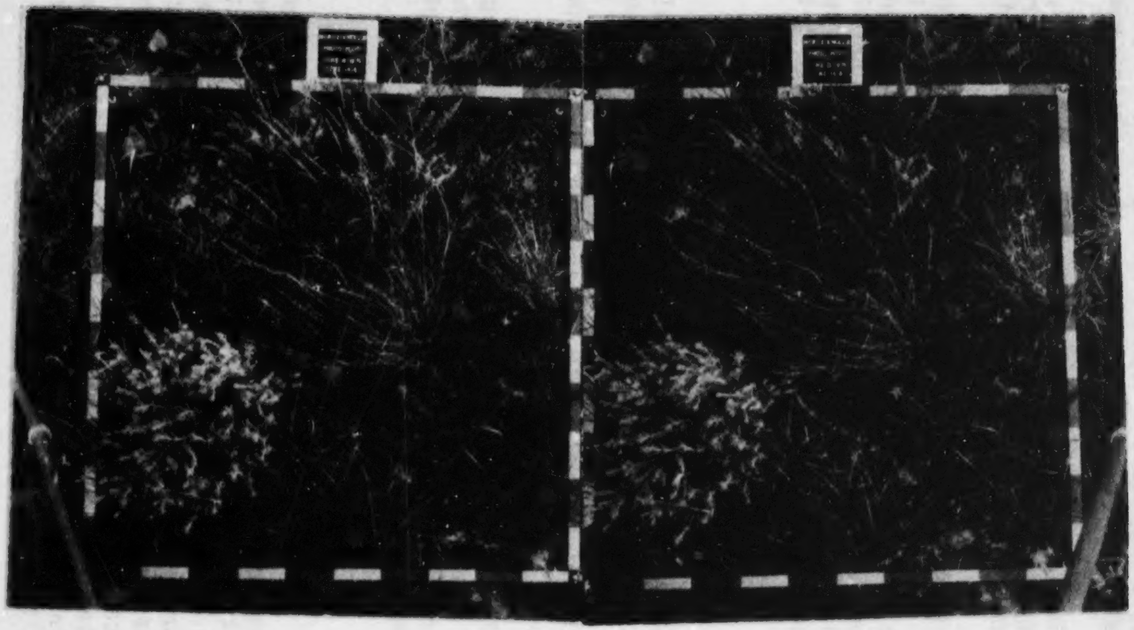
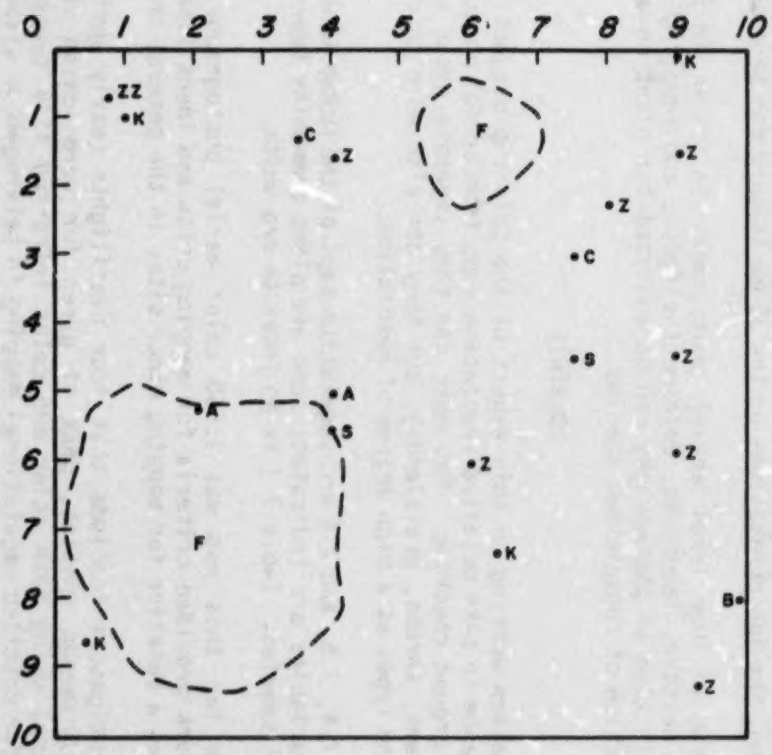


Figure 7.1. Example stereogram of photoplot (McRae Knoll B, June 16, 1975).



| SYMBOL | SPECIES PRESENT |
|---|---|
| — | *Agropyron spicatum* * |
| X | *Allium textile* |
| Z | *Artemisia cana* |
| C | *Artemisia frigida* |
| — | *Carex filifolia** |
| F | *Ceratoides lanata* |
| — | *Koeleria cristata** |
| K | *Leucocrinum montanum* |
| S | *Sphaeralcea coccinea* |
| — | *Stipa comata** |
| B | *Taraxacum officinale* |
| A | *Tragopogon dubius* |

(*MATRIX SPECIES)

Figure 7.2. Photoplot index of McRae Knoll B, June 17, 1975.

## DISCUSSION

Three primary film types are used with other materials for special purposes. The main films are color, color infra-red and black-and-white (H&W VTE Pan). Each has advantages for particular uses.

Color is realistic and many plant species are easily recognized. It is easy and fast to process, and is visually acceptable to lay viewers.

Color infra-red is excellent for delineating plant species and stress effects. Although it appears strange to inexperienced users, it is useful for many aspects of this project.

Black-and-white is most practical for mapping photography, especially at relatively small scales. It makes a good, inexpensive base map for overlaying ground information (vegetation, geologic features, soil types, drainages, etc.).

The Hasselblad format (6x6 cm) allows greater coverage than the 35 mm (2.4x3.6 cm) frame. Especially at low elevations (<1000 ft), this increases the chance of covering ground targets completely. The 35 mm photography permits processing in the field, so that pictures taken one day can be interpreted the next. Also, if exposures or coverage are not acceptable, a reflight can be made immediately. When the larger format photography is returned from processing, the interpretations on the 35 mm imagery can be applied.

An example of low level aerial photography is given in Figure 7.3. General plant pattern, landforms, deferment effects, and sampling impacts are apparent. This kind of photography can be analyzed for plant cover, pattern, and identification of conspicuous species.

## COLSTRIP

Investigators working on this aspect of the Colstrip project have gained enough experience to make detailed vegetation maps from aerial photographs with only limited ground checking. Not only can they separate gross vegetational groupings (trees, shrubs, grasslands), but they are also able to discriminate among grassland types at a high degree of resolution.

Figures 7.4, 7.5, and 7.6 are vegetation maps of the McRae Knolls. Thirty-six mapping variables are indicated; some are plant community types and others are individual species. Table 7.1 is an index to map units.

The base for this map was 1:660 color aerial photography. A ground transect network provided criteria for mapping units and identification. This map constitutes a baseline for mapping other sites in the general area.

The investigators conclude that four overflights (early spring, peak of green for cool-season species, peak of green for warm-season species, and summer dormancy) using both color and color infra-red film types provide an adquate base for detailed vegetational mapping in heterogenous situations.

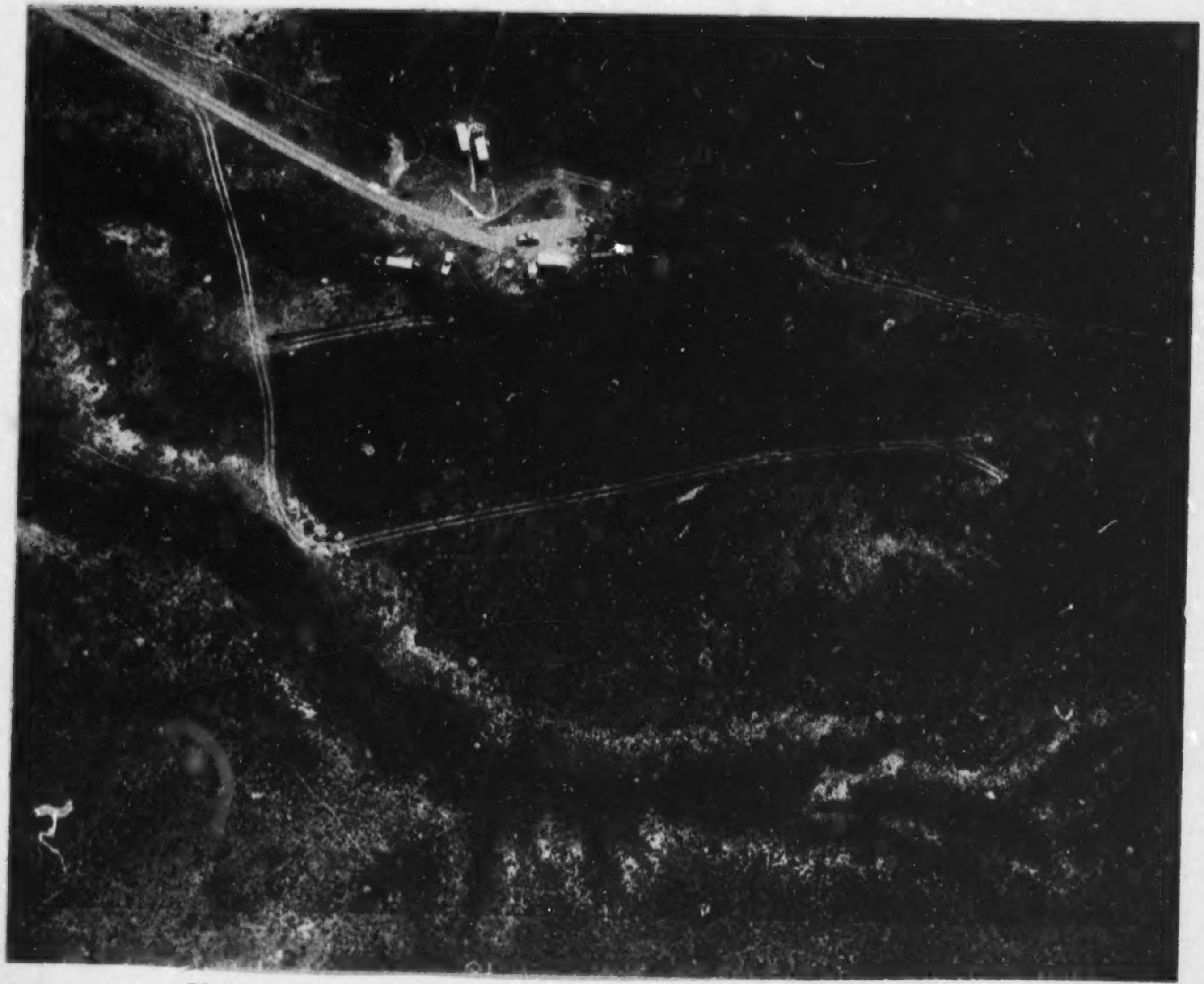


Figure 7.3. Low level aerial photograph of Hay Coulee exclosure.

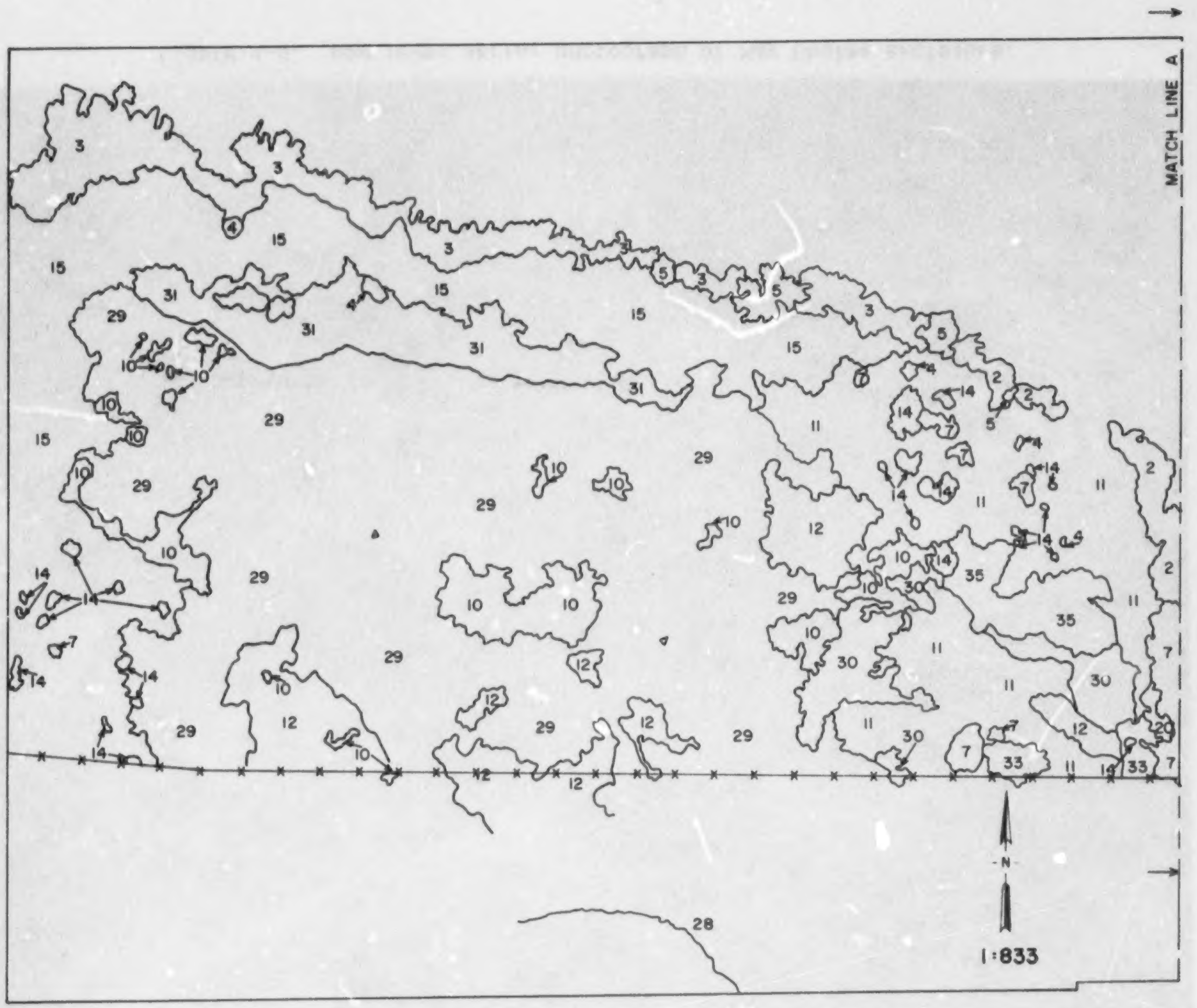

MATCH LINE A
N
1:833

Figure 7.4. Detailed vegetation map, McRae Knoll A, Summer, 1976.

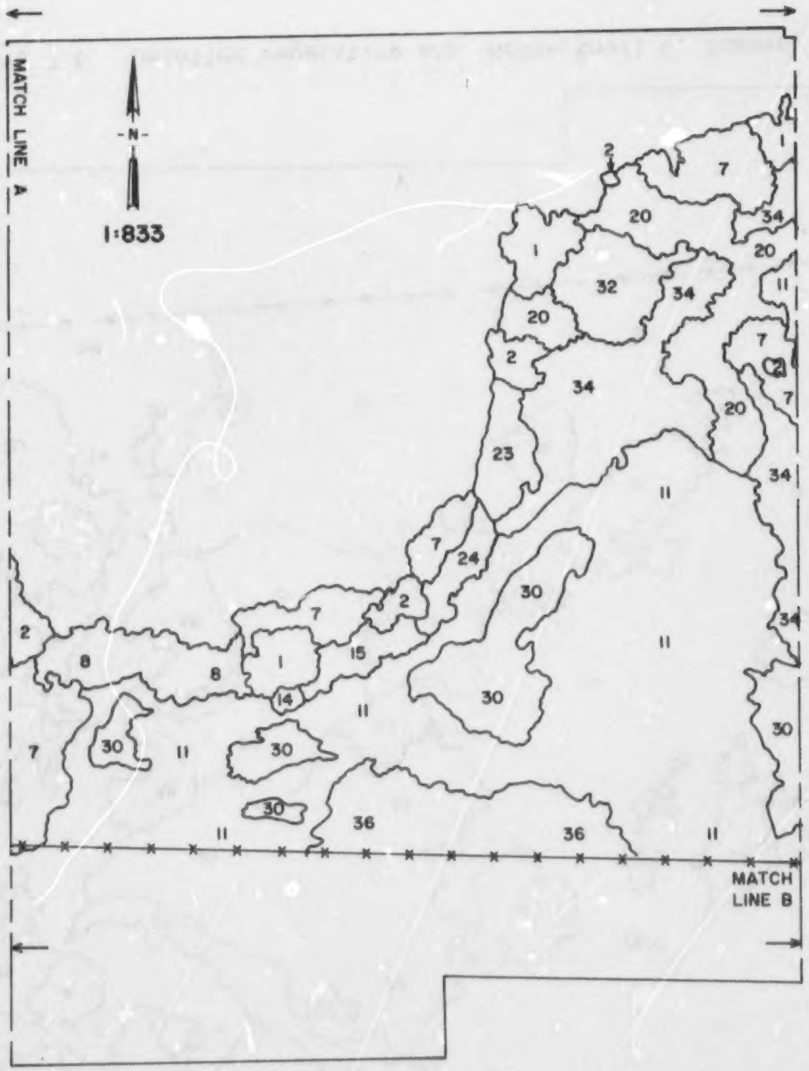

MATCH LINE A
-N-
1:833
MATCH LINE B

Figure 7.5. Detailed vegetation map, McRae Knoll B, Summer, 1976.

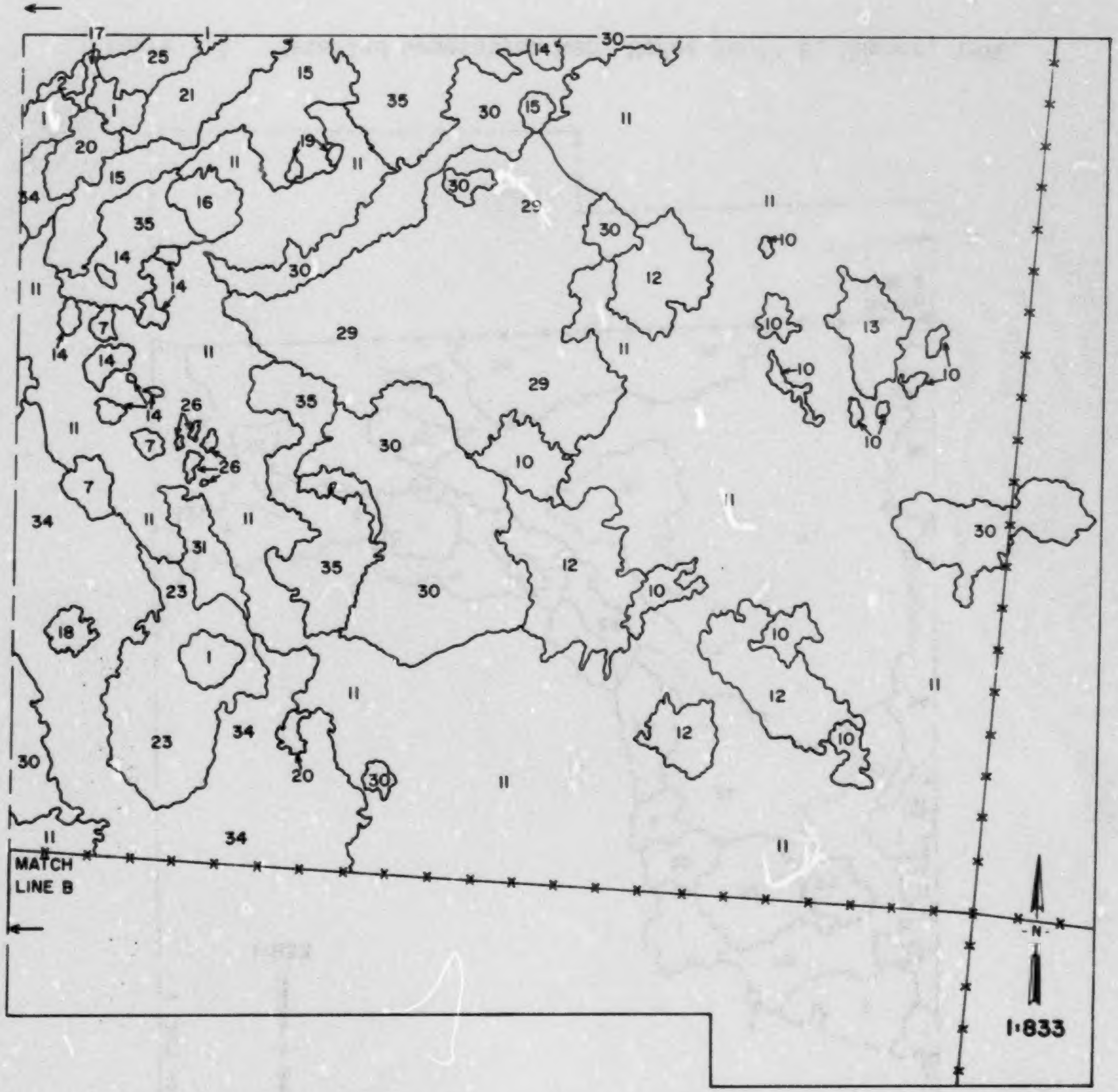

MATCH
LINE B
N
1:833

Figure 7.6. Detailed vegetation map, McRae Knoll C, Summer, 1976.

TABLE 7.1. INDEX TO MAP UNITS.

| SYMBOL | COMMUNITY |
|---|---|
| *Tree types* | |
| 1 | *Acer negunda* |
| 2 | *Fraxinus pennsylvanica* |
| 3 | *F. pennsylvanica/Prunus virginiana* |
| 4 | *Juniperus scopulorum* |
| 5 | *Populus deltoides* |
| 6 | *Prunus americana* |
| 7 | *P. virginiana* |
| 8 | *Prunus/Acer/Fraxinus* |
| 9 | *Salix amygdaloides* |
| *Shrub types* | |
| 10 | *Artemisia cana* |
| 11 | *A. cana/mixed grass* |
| 12 | *Artemisia dracunulus* |
| 13 | *Ceratoides lanata* |
| 14 | *Rhus trilobata* |
| 15 | *R. trilobata/P. virginiana* |
| 16 | *R. trilobata/Ribes/P. virginiana* |
| 17 | *Ribes* |
| 18 | *Rosa woodsii* |
| 19 | *Shepherdia canadensis* |
| 20 | *Symphoricarpos occidentalis* |
| 21 | *S. occidentalis/A. cana* |
| 22 | *S. occidentalis/Ribes* |
| 23 | *S. occidentalis/Ribes/R. woodsii* |
| 24 | *S. occidentalis/R. woodsii* |
| 25 | *S. occidentalis/R. woodsii/Ribes/P. virginiana* |
| *Forb types* | |
| 26 | *Yucca glauca* |
| 36 | *Melilotus officinalis* |
| *Grass types* | |
| 27 | *Bromus inermis* |
| 28 | *B. japonicus/B. tectorum* |
| 29 | *Carex filifolia/B. japonicus* |
| 30 | *Calamovilfa longifolia* |
| 31 | *C. longifolia/C. filifolia* |
| 32 | *Poa pratensis/Agropyron smithii/Stipi viridula* |
| 33 | *P. pratensis/Carex* |
| 34 | *P. pratensis/S. viridula/A. cana/A. smithii* |
| 35 | *Schizachyrium scoparium* |

In more homogenous vegetation, additional ground studies are needed to elucidate the more subtle community patterns. The Kluver sites and Hay Coulee fall in this category. Larger scale photography and further ground observations are planned for these locations.

## ZAPS

Even though the aerial photography shows distinct tonal differences among the ZAPS plots, the reasons are still to be determined. Early signs of $SO_2$ stress can be detected, but the magnitude of pollution damage cannot be quantified. Additional ground studies and more detailed photographic analysis should lead to an accurate and fast method for damage assessment.

## SUMMARY

Photo plots have been established throughout the study area, and are examined periodically during the growing season. They provide records or changing species composition, cover, and stress effects.

Aerial photography techniques have been developed to monitor air pollution effects as shown by plant changes. Color, color infra-red and black-and-white films each have particular uses.

Pollution stress is detectable in aerial photography, but cannot be quantified without additional analysis.

A procedure for vegetational mapping has been developed and tested.

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Woodcock, W. E. 1976. Aerial reconnaissance and photogrammetric with small cameras. Photogrammetric Eng. and Remote Sensing.42:503-511.

# SECTION 8

# INTEGRATED AEROSOL CHARACTERIZATION MONITORING, COLSTRIP, MONTANA

by

N. L. Abshire, V. E. Derr, G. T. McNice, R. Pueschel, and C. Van Valin

## INTRODUCTION

### GENERAL

In addition to other objectives, the Colstrip Coal-Fired Power Plant Project requires the concentration, dispersion and identification of background particulate matter and particulate matter resulting from the activities of the mines and coal-fired power plants. This project started in May 1975. Observations were performed at Hay Coulee from May 18 to June 16, 1975; from August 17 to September 15, 1975; and from May 23 to June 5, 1976. In addition, aircraft flights were made on April 20 and 21, 1976. The observation periods were chosen to give maximum density of observation in the Spring and Fall at the beginning and end of the growing season. Beginning in October 1976 a sampling flight was made between the intense observation periods. Further intense observations were made August 14 to August 28, 1976, and in the Spring and Fall of 1977.

This research program is designed to help the Environmental Protection Agency Laboratory at Corvallis, Oregon, assess the impact of coal-fired power plants on a grassland ecosystem and develop a valid and effective environmental impact assessment protocol. Atmosphere particles emitted by coal-fired plants (e.g., ash) or formed from gases emitted (e.g., sulfates or chlorides) produce an array of effects on the ecosystem. These particulates may produce direct biolgical effects by contact through leaves and soil. They may also affect the radiation reaching the biosphere by absorption and blanketing mechanisms. Further, by adding cloud condensation nuclei and ice nuclei to the atmosphere, they may significantly modify precipitation, either near the source or far downwind. The effects of particulate matters are strongly dependent on the temperature structure (inversions) and mixing and diffusion characteristics of the atmosphere downwind from the source. The parameters needed to characterize the effects of particles should be examined over a wide geographical area and over sufficient time to obtain representative averages.

The program seeks to characterize the impact of air pollutants on the total ecosystem. Small particles are a significant part of the pollution emitted by fossil fuel power plants, even when the stack emission has been carefully filtered. These particles from the stacks and the natural background of particles from the earth's surface are called "aerosols." For this report, clouds and fogs are excluded from the term aerosols. Aerosols range in size from a clump of a few molecules to tens of micrometers. The larger aerosol particles fall out rapidly and are not as frequently observed in the atmosphere. However, particles up to several micrometers in diameter may remain suspended in the atmosphere for long periods, up to weeks or months. Thus if filters which remove large particles are not as effective in removing small particles, the plume from a smoke stack may produce significant pollution which can remain suspended in the vicinity, or be swept by winds over distances up to several hundred miles. In order to evaluate the amount of such material, its source, its distribution in the atmosphere, the effects of local weather conditions and terrain, and to identify the composition and size and shape distributions of the particles, it is necessary to use special remote, in situ and airborne sensors. These sensors are described in some detail in the Discussion section of this paper. The principles of such measurements are discussed below.

## MEASUREMENT RATIONALE

### Particles

The remote measurement of the distribution of particles in the atmosphere is accomplished by the use of lidar (Light Detection and Ranging). Lidar operates very much like radar. A powerful short pulse of light ($10^{-8}$sec) is transmitted. The light scattered back from small particles is collected by a telescope (70 cm diameter) and detected by photomultipliers. Lasers, operating at near visible wavelengths are required to detect particles whose radii are of approximately the same size as the electromagnetic radiation (light) waves scattered. If the particles are small compared with the wavelength of the radiation (as would be the case for microwave radar) the scattered radiation is too weak to detect. Detection of particles by a calibrated lidar yields two pieces of information. First, the backscatter cross section is obtained. It is dependent on the size, shape and index of refraction of the particles and is proportional to the number of particles of a given size, shape and index. The chief asset of lidar remote sensors is the ability to obtain signals from particles at large distances, up to 20 km, and by scanning the vertical hemisphere, with good spatial resolution, to determine the distribution of particles. Second, the polarization of the scattered light is measured. Since the laser transmitter is linearly polarized, the degree of depolarization caused by the scatterer may be determined.

The degree of depolarization is indicative of the shape of the particle: spherical particles produce no depolarization in backscatter; non-spherical particles can cause significant depolarization. Since newly-disturbed dust particles in the atmosphere are frequently very non-spherical, the depolarization is frequently a useful way of discriminating between dust from the mines and the more spherical particles from the stacks.

The lidar cannot measure mass or particle concentrations without calibration. For that purpose, aircraft sampling is employed. Suitable nucleopore or millipore membrane filters capture particles for later elemental, size, and shape analysis with an x-ray fluoresence spectrometer and an electron microscope. The aircraft also contains an Aitken particle counter which determines the number of particles per liter. With these calibrations, and since the variation of aerosol types in time and space is small compared with aircraft sample times, the lidar may be used efficiently to measure mass concentrations over very large volumes and observe changes in those concentrations under the influence of local weather conditions.

The very important identification of particle characteristics is performed by several instruments located on the ground in a trailer next to the lidar system. This system, of fundamental importance to the project, measures many parameters of the particles. The trailer is equipped to continuously measure the total aerosol population, or "Aitken nuclei," (active at 300 percent supersaturation), cloud condensation nuclei (active at 0.1 percent supersaturation), aerosol light scattering and standard meteorological parameters such as wind speed, direction, temperature, and humidity.

The direct measurement of solar radiation at visible wavelengths can provide an independent determination of aerosol loading. Radiation from the sun in the visible range is known to remain constant to within 1 percent over periods of decades. Observation of solar irradiation by instruments at ground level can therefore be used to accurately measure the attenuation imposed by the atmosphere. The major causes of atmospheric attenuation (turbidity) are clouds, aerosols, ozone, water vapor, and Rayleigh scattering by the air. The effect of Rayleigh scattering can be accurately calculated and allowed for. Ozone and water vapor absorption occurs in specific wavelength bands and can thus be avoided or corrected by proper choice of wavelength band for the observing equipment. Therefore, when clouds are not between the sun and observer, the attenuation by atmospheric aerosols alone can be determined.

## Radiation Effects

Two radiation measurements are of importance. First, the rate at which solar energy reaches the earth as a function of wavelength must be measured while the power plants are not in operation (baseline measurements). The changes registered during succeeding measurements while the plants are operating may then be correlated with the particulate loading and changes in cloud cover. Second, the net heat loss of the earth must be measured by infrared radiometry by measuring the upward and downward radiation, employing aircraft and ground-based instruments, operating from 8 to 12 μm.

Measuring the IR fluxes within and outside the plumes gives the IR "shadowing" effect by the plume, inversion techniques permit the determination of the IR volume absorption coefficient. In conjunction with the measured aerosol size distribution, the aerosol absorption index can be evaluated, aiding in the evaluation of long-term climatic effects.

## Meteorological Conditions

Standard meteorological variables are measured by standard instruments. The meteorological conditions which trap pollutants below temperature inversions are best measured by an acoustic sounder. The acoustic sounder is also a radar-like device, which uses a pulse of sound. The scattering of sound back to the receiver is dependent on the degree of homogeneity in vertical temperature structure. The device is especially sensitive then to temperature inversions and can determine their height and persistence.

## ENVIRONMENTAL EFFECTS

Environmental and health effects of aerosols produced by human activities and by industrial activities in rural Montana are foreseen in four areas:

1. Inadvertent weather modification: Size, shape and water solubility (chemical composition) determine the cloud and ice nucleating capabilities, and thereby effects of the atmospheric aerosol on cloud and precipitation processes. The proposed experiments are designed to scrutinize a cause-effect relationship between weather modifying particular matter and human activities in Montana.

2. Climate modification: Size, shape and chemical composition (refractive index) determine the light scattering and absorption capability of the atmospheric aerosol. The backscattering to absorption ratio, in relation to the surface albedo, is the critical parameter that determines whether the aerosol's effect leads to cooling or warming of the earth-atmosphere system. IR extinction can cause surface temperature changes by thermal blanketing and affect radiometric measurements from satellites. All of these parameters will be evaluated by means of the experimental data.

3. Ecological effects: The damage to vegetation by sulfur and halogen gases and to structures by corrosive particulate sulfates and halides downwind from their sources is greatly affected by the gas-to-particle conversion rate. In situ measurements of this conversion rate as function of the amount of water vapor and third-body aerosols is planned by adding proper gas analysis techniques to the measurements proposed.

4. Health effects: Particle sizes determine the depth of penetration of an aerosol into the animal respiratory system. Particle solubility (chemical composition) determines their retention times. The data from the proposed research will enable health officials to better evaluate health hazards resulting from environmental pollution.

## GENERAL DISCUSSION OF OBSERVATIONS

Observations were planned for Spring and late Summer of 1975 to provide a baseline against which to judge changes in atmospheric aerosols after the coal-burning plants began operation. Further observation was planned for Spring and late Summer 1976 and 1977. The observation of May 23 to June 15, 1976, provided opportunity only for measurements of unit #2 operating under initial tests with gas rather than coal. Interesting differences from 1975 observations were observed which can only be partially reported here until the data processing is complete.

# MEASUREMENT TECHNIQUES

## GENERAL APPROACH

In order to adequately characterize the aerosol content, a combination of observation techniques was used. The method was to make well calibrated in situ and airborne measurements to provide benchmarks for some of the remote sensing devices, primarily the lidar (laser radar), enabling them to extend the calibrations throughout the volume of interest. The various devices and techniques are described below and are summarized in Tables 8.1 and 8.2.

## SURFACE MEASUREMENTS

1. Aerosol collection: Aerosols were collected on membrane filters and later examined for number, shape and size distribution in a transmission electron microscope, for elemental composition (atomic number $\geq$ 11) in a scanning electron microscope with energy dispersive x-ray analysis, and for ice nuclei in a thermal diffusion chamber.

2. Aitken Nuclei (AN) count: AN are defined as the total aerosol population, and their concentration was measured with a continuously operating Environment-One particle counter. This unit subjects a humidified air sample to rapid adiabatic expansion that results in a water saturation of 300 to 400 percent. In this condition, water droplets form on all aerosol particles present in the chamber. The particle concentration is proportional to the attenuation of a light beam propagated through the cloud.

3. Cloud Condensation Nuclei (CCN): Many of the AN are effective as CCN. Their concentration was measured in a thermal diffusion chamber by the photographic technique of Allee (1974) at .5 percent supersaturation, a valve typically found in nature. The cloud droplets found were photographed and counted.

4. Ice Nuclei (IN): IN comprise a small but important subset of the AN. These were counted with an NCAR acoustic ice nucleus counter (Langer et al., 1967) at -20°C (Bigg et al., 1963).

5. Gas analysis: Sulfur dioxide was monitored with a Meloy Sulfur Gas Analyzer. A Theta Sensors, Inc. sulfur dioxide analyzer was used for the 1976 airborne measurements.

6. Meteorology: Wind speed and direction, temperature, relative humidity and insolation were measured continuously.

7. Insolation: The total radiant energy density, including both direct and scattered sunlight, was continously monitored with an Eppley pyranometer.

8. Light scattering: The coefficient of scattering of atmospheric aerosols was measured continuously with the Meteorology Research, Inc. model 1550 nephelometer.

TABLE 8.1. PARAMETERS MEASURED IN THE NOAA EPA COLSTRIP INTEGRATED ATMOSPHERIC CHARACTERIZATION PROGRAM.

| Parameter | Units | Method of Recording | Instrument | Frequency of Recording | Precision | Accuracy | Remarks |
|---|---|---|---|---|---|---|---|
| Temperature | °C | Recorder | — | 60, (00) | 0.5 C | 0.5 C | — |
| Wind, Direction, Vel. | M/S, Degrees | Recorder | — | Continuous | 10% | 10% | — |
| Cloud Cover | % | Log | — | 1. 2 times daily or when changes occur<br>2. Whenever insolation meas. made | See FMH - 1 | | — |
| Relative Humidity | % | Recorder | — | Continuous | 10% | 10% | — |
| Solar Energy, Direct | Cal/cm$^2$/min | — | Pyrheliometer (no filters) | Continuous during day | 5% | 5% | Wide Band |
| Solar Energy, Direct Spectral | Cal/cm$^2$/min | — | Pyrheliometer (w/3 filters) | 1000, 1300, 1600 | 5% | 5% | 3 wavelength bands, when sun's disk is visible |
| Solar Energy, Total | Cal/cm$^2$/min | — | Pyranometer (no filters) | Continuous during day | 5% | 5% | APCL + WPL |
| Turbidity | Volts | Log | Volz Turbiditymeter | 1000, 1300, 1600 | Unknown | Unknown | — |
| Lidar Aerosol Scatter | Backscatter Intensity | Log, Digital Tape, Photos | Lidar System | In accordance with variability | ±10% | ±30% | Polarization Ratio Available |
| Radar | Backscatter Intensity (Uncalibrated) | Photos | Radar System | Clouds only | — | — | Test for Cloud Observation |
| Atmospheric Temperature Turbulence | Backscatter Intensity (Uncalibrated) | Facsimile | Acoustic Sounder | Continuous | — | — | Mark time on Facsimile Chart |
| Radiometer | watts/cm$^2$ | Chart, Tape | Barnes PRT-5 | Chart-continuous tape-on each shot | 10% | 10% | Mark time on Chart |
| Photography | — | Film | Camera | As needed | — | — | Targets of Opportunity |

TABLE 8.2. PARAMETERS MEASURED IN THE NOAA EPA COLSTRIP INTEGRATED ATMOSPHERIC CHARACTERIZATION PROGRAM. (continued)

| Parameter | Units | Method of Recording | Instrument | Frequency of Recording | Precision | Accuracy | Remarks |
|---|---|---|---|---|---|---|---|
| Cloud Condensation Nuclei | $CM^{-3}$ | Recorder | MRI | Continuous | ±20% | Unknown | — |
| Cloud Condensation Nuclei | $CM^{-3}$ | Photographic | Allee Photographic | Point Sample | ±10% | Unknown but high | Visual Counting–Absolute Measurement |
| Ice Nuclei | $CM^{-3}$ | R | NCAR Acoustic Counter | C | — | Unknown (State of the Art) | — |
| Ice Nuclei | $CM^{-3}$ | L | Langer Diffusion Chamber | PTI | ±50% | Unknown (State of the Art) | Including Airborne Collection by Membrane Filter |
| Light Scattering | $b_{scat}(10^{-4}m^{-1})$ | R | MRI | C | ± 5% | ± 5% | — |
| Aitken Nuclei | $CM^{-3}$ | R | Env.-1 | C | ±10% | ±10% | — |
| Aitken Nuclei | $CM^{-3}$ | L | Gardner Counter | Point Sample | ±10% | — | Ground and Airborne Measurements |
| Cloud Cover (Insolation) | $CM^{-3}$ | R | Eppley Pyranometer | C | — | — | — |
| Sulfur Gases | ppb-ppm | R | Meloy | — | ±0.01 ppm | ±0.01 ppm | Including Airborne Measurements |
| Elemental Composition Of Individual Particles | — | P and L | Electron Microscope (SEM-EDX) | Preselected Time Intervals | For Heavy Metals Approximate Sensitivity $10^{-15}$gm | — | Membrane Filter Collection–Ground and Airborne |
| Aerosol Size Distribution | — | P and L | Electron Microscope (SEM-EDX) | Preselected Time Intervals | ±10% | ±10% | Membrane Filter Collection–Ground and Airborne |
| Atmospheric Transmissivity (IR Extinction Coefficient) | — | R | Barnes Radiometer | Airborne Measurements | 0.5 C | 0.5 C | Downward Looking |

## AIRBORNE MEASUREMENTS

A Cessna 182 single engine aircraft was utilized for the measurement of temperature, AN concentration (using a Gardner Small Particle Counter) and the collection of aerosols on membrane filters as described earlier. A Barnes PRT-5 radiometer was utilized for the measurement of atmospheric transmissivity looking down.

## REMOTE SENSING MEASUREMENTS

1. Lidar: The remote measurement of atmospheric particle density was accomplished through the use of Lidar. A short powerful pulse of light is transmitted into the atmosphere. Some of this light is scattered by the atmosphere back into a telescope, detected and processed, yielding the optical backscatter coefficient of the scatterer. This quantity is proportional to the number of scatterers of given size, shape and refractive index, but cannot produce either mass concentration, size distribution or chemical composition without the support of the in situ and airborne measurements. For a given situation, however, it is very sensitive to any changes, particularly in concentration, and has proved very useful in alerting the other observers as to a change in the character of the atmospheric aerosols.

The lidar system consists of a semi-trailer and a van (Figure 8.1). The lasers and telescope are located in the semi-trailer. The laser generates a 5 joule pulse, at 6943 Ångstroms wavelength, 30 nanoseconds in duration at repetition rates up to 1 pulse per second. The beam is collimated to 1 milliradian beamwidth and aligned parallel to the telescope within 1 milliradian. The telescope is a folded Newtonian configuration with a 70 cm diameter and a 2 meter focal length, and can be positioned to cover the vertical hemisphere. The backscattered light is collected in the telescope, transmitted to photomultipliers, detected and sent to the van for processing.

The detected signal is digitized every 10 nanoseconds and stored on magnetic tape for data reduction in a large digital computer. The digitizer also stores the signal return and presents it on an oscilloscope for real time evaluation of the raw data. Auxiliary information such as telescope position, date and time, laser power, and photomultiplier voltage are recorded along with the data.

The lidar system is calibrated through the use of an 8 x 8 foot target of known optical properties. These properties (reflectively, uniformity, polarization properties) are measured in the laboratory before and after each field trip.

2. Acoustic Sounder: The degree of pollutant trapping is strongly influenced by the vertical temperature structure, particularly the existence, height and strength of a temperature inversion layer. The vertical temperature structure was continuously monitored by an acoustic sounder of the type described by Owens (1975). This unit also operates in a fashion similar to radar. It emits a 100 watt, 100 millisecond sound pulse every 10 seconds in a vertical beam. Variations in temperature scatter some of the sound back into a five foot acoustic collector, where it is detected and presented on a

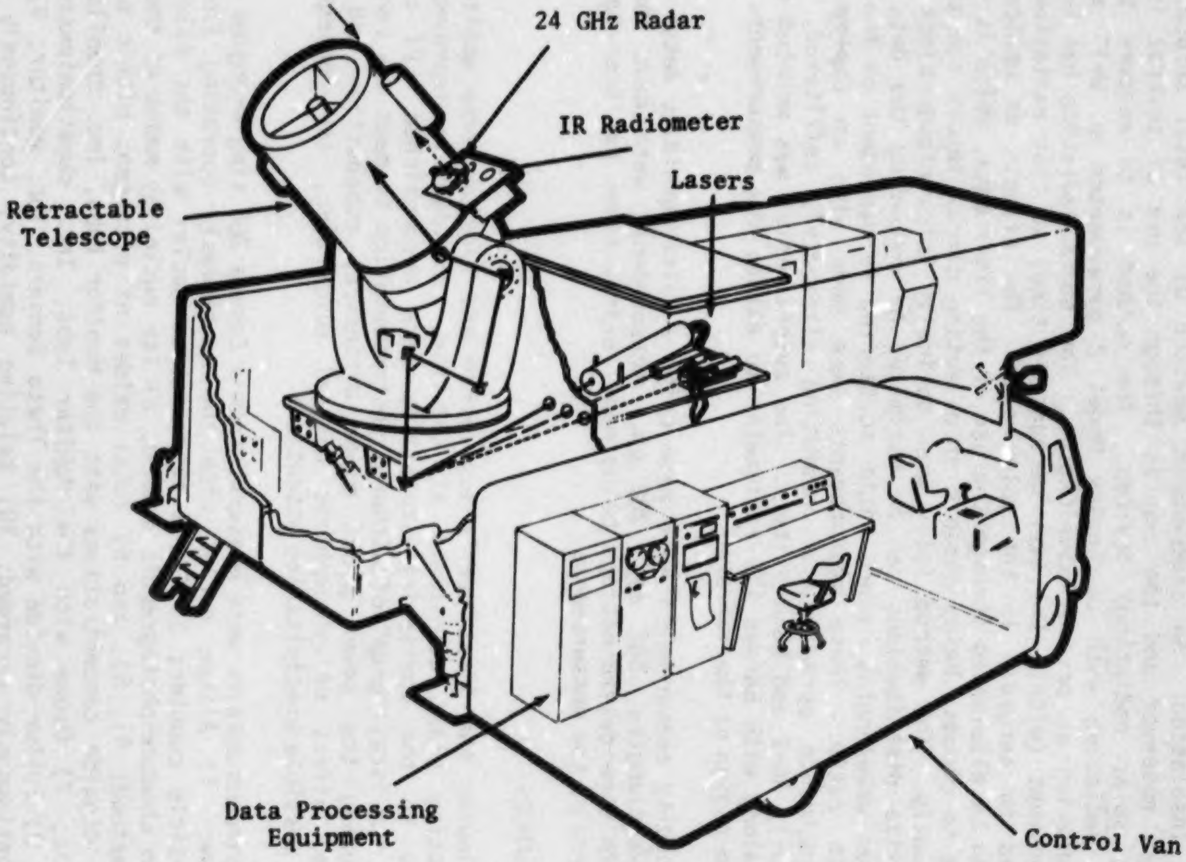
24 GHz Radar
IR Radiometer
Lasers
Retractable Telescope
Data Processing Equipment
Control Van

Figure 8.1. NOAA lidar system.

facsimile chart. This information has proved very useful in the location of inversion layers, so that the aircraft could be properly positioned for maximum utility. Quantitative information regarding the strength of the inversion was not obtained.

3. Insolation: An independent measure of the total aerosol content between an observer and the sun is through the use of several insolation (incoming solar radiation) devices. The method is to measure the total incident radiation with an Eppley Model 2 pyranometer as well as direct solar radiation at several wavelengths. The sun's radiation has been shown to be constant (within 1%) over decades of time, so that variations can be attributed to aerosols in the solar path. The effects of optically thin clouds can be eliminated through the use of the lidar data, which is extremely sensitive to clouds, being capable of detecting cirrus layers too thin to be seen visually. The method fails when optically thick clouds block the sun. Aerosol size distributions can be estimated by comparing the data obtained at various wavelengths, since light scattering is dependent on the size-to-wavelength ratio. These measurements were made with an Eppley Model 8 normal incidence pyrheliometer operating alternately unfiltered, and with Schott OG-1, RG-2 and RG-8 filters. The pyrheliometer was mounted on a sun-tracker along with Barnes IT3 radiometer to extend the measurements into the 8 to 12 µm region of the infrared.

Turbidity measurements were made using a Voltz turbidity meter operating at the wavelengths .502 and .382 µm. Measurements were made three times daily with some determinations being made several times per hour to test the consistency of the measurements.

## RECENT CHANGES

Following the first year's experience and findings from monitoring the pregenerating plant atmosphere at Colstrip, considerable improvements and additions to the monitoring capability were accomplished. Of particular note was the "scaling-up" of airborne instrumentation to meet the requirements of measuring the power plant plume diffusion, composition and chemical reactions, effect on atmospheric optical properties, and consequences in terms of possible precipitation attention.

Eleven parameters were measured from a Cessna 206 single-engine airplane. These are: 1) Aitken nuclei with the continously operating Environment, Inc. particle counter; 2) Cloud condensation nuclei with the Allee thermal diffusion chamber-photographic method; 3) Ice nuclei by means of the membrane filter method; 4), 5), and 6) Total oxides of nitrogen, nitric oxide, and nitrogen dioxide concentrations with the Monitor Labs, Inc. chemiluminescent instrument; 7) Ozone with the Monitor Labs, Inc. chemiluminescent ozone monitor; 8) Sulfur dioxide with the Theta Sensors, Inc. monitor; 9) Temperature, continuously recorded; 10) Relative humidity, continuously recorded; and 11) Light scattering with the Meteorology Research, Inc. continuously operating Model 1550 nephelometer. In addition, membrane filter collections of atmospheric aerosols were done for subsequent analysis by Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM). The SEM with energy dispersive x-ray analysis, yields the elemental composition of individual particles of diameter $\geq$ 0.1 μm, and for particle topography. The TEM is utilized for the aerosol size distribution, which is crucial to calculations of possible atmospheric heating or cooling, and transmissivity.

The mobile laboratory was operated in the same location as for the previous year's study. Parameters measured were: 1) Sulfur dioxide with the Meloy Laboratories, Inc. flame photometric sulfur gas analyzer; 2) Atmospheric light scattering with the Meteorology Research, Inc. nephelometer; 3) Ice nuclei by the NCAR acoustic counter method (continuous); 4) Ice nuclei by the membrane filter method; 5) temperature; 6) relative humidity; 7) wind speed; and 8) wind direction. Membrane filters were also collected according to a pre-determined schedule, for examination by SEM and TEM. Pilot balloon releases and tracking were done once each hour for wind direction and velocity aloft while the aircraft was operating.

The overall measurement capabilities are summarized in Tables 8.1 and 8.2.

## DISCUSSION

### LIDAR DATA

The system was on site for 30 days in the Spring (May 18 through June 16) and the Fall of 1975. The Spring was an unusually wet one, with some rainfall occurring nearly every day. The Fall was more typical--mostly hot and dry with occasional showers. The power plant was not in operation during either period, so the data gathered will be used as a baseline for future measurements.

Figure 8.2 is representative of the lidar data for the Spring period. The signal begins at zero as the laser beam is initiated outside the telescope's field of view. As the beam comes within the field of view the signal increases until all of the beam is totally contained and is said to have passed "crossover." Data recorded at heights lower than crossover are not valid, since not all of the laser energy is used. This curve indicates a nearly uniform atmosphere with an aerosol loading approximately 15 times a theoretically clear atmosphere. The aircraft was operating 900 meters above the surface when these lidar data was recorded and the optical backscatter coefficient calculated from the particles collected in the membrane filter at this altitude is also plotted.

Figure 8.3 shows a typical acoustic sounder record for the 1975 Spring experiment. A weak inversion structure is seen operating from 0000 to about 0900 when thermal activity becomes the dominant feature. The dark bands from 1200 to 1400 are due to the aircraft operating overhead. Thermal activity continues until 2000 when the evening temperature inversion reappears. The Spring inversions, if present, were usually very weak, and would not have played a significant role in the trapping of pollutants.

Figure 8.4 is representative of the 1976 Fall lidar data. It is essentially the same as the Spring data except for a sharp layer at a height of 500 meters due to aerosols trapped by a weak inversion layer. The corresponding acoustic sounder record (Figure 8.5) barely shows the temperature inversion.

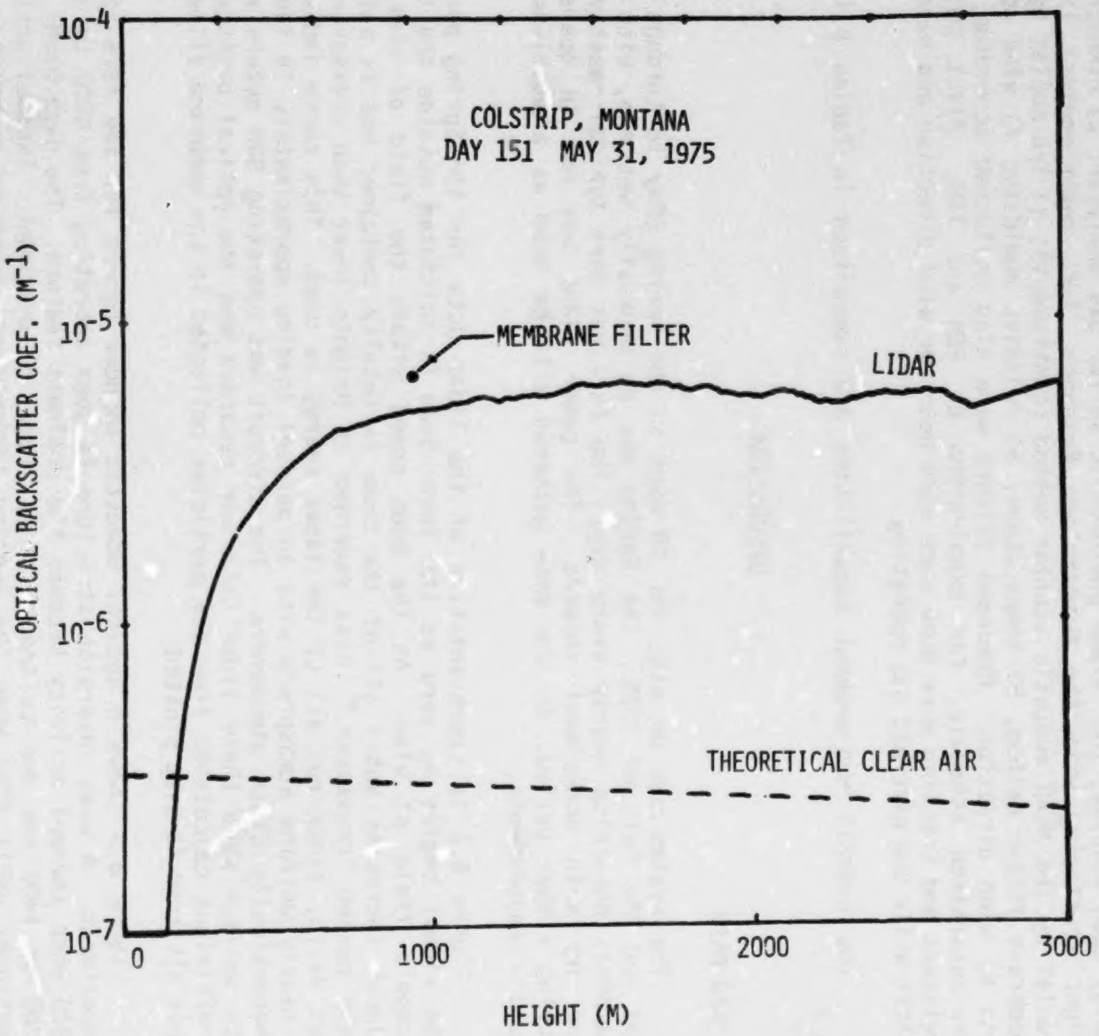

Figure 8.2. Optical backscatter--Spring 1975.

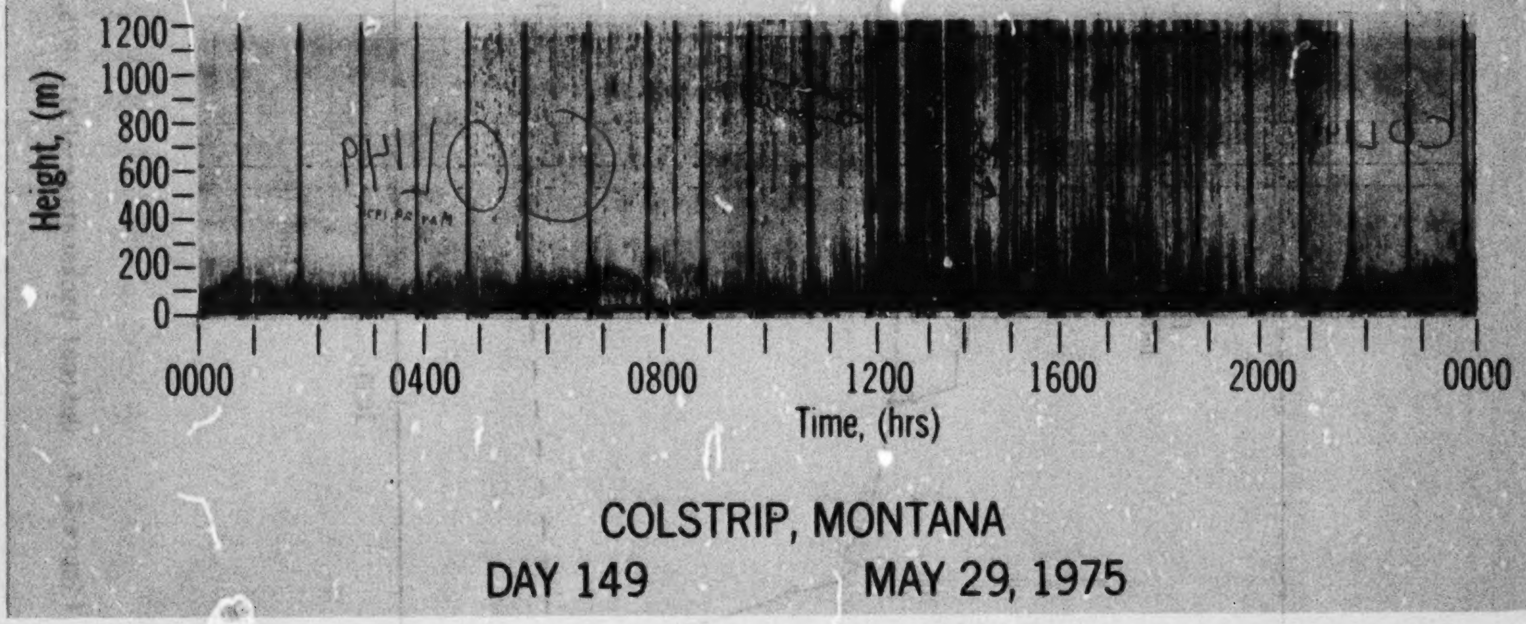

Figure 8.3. Acoustic sounder record--Spring 1975.

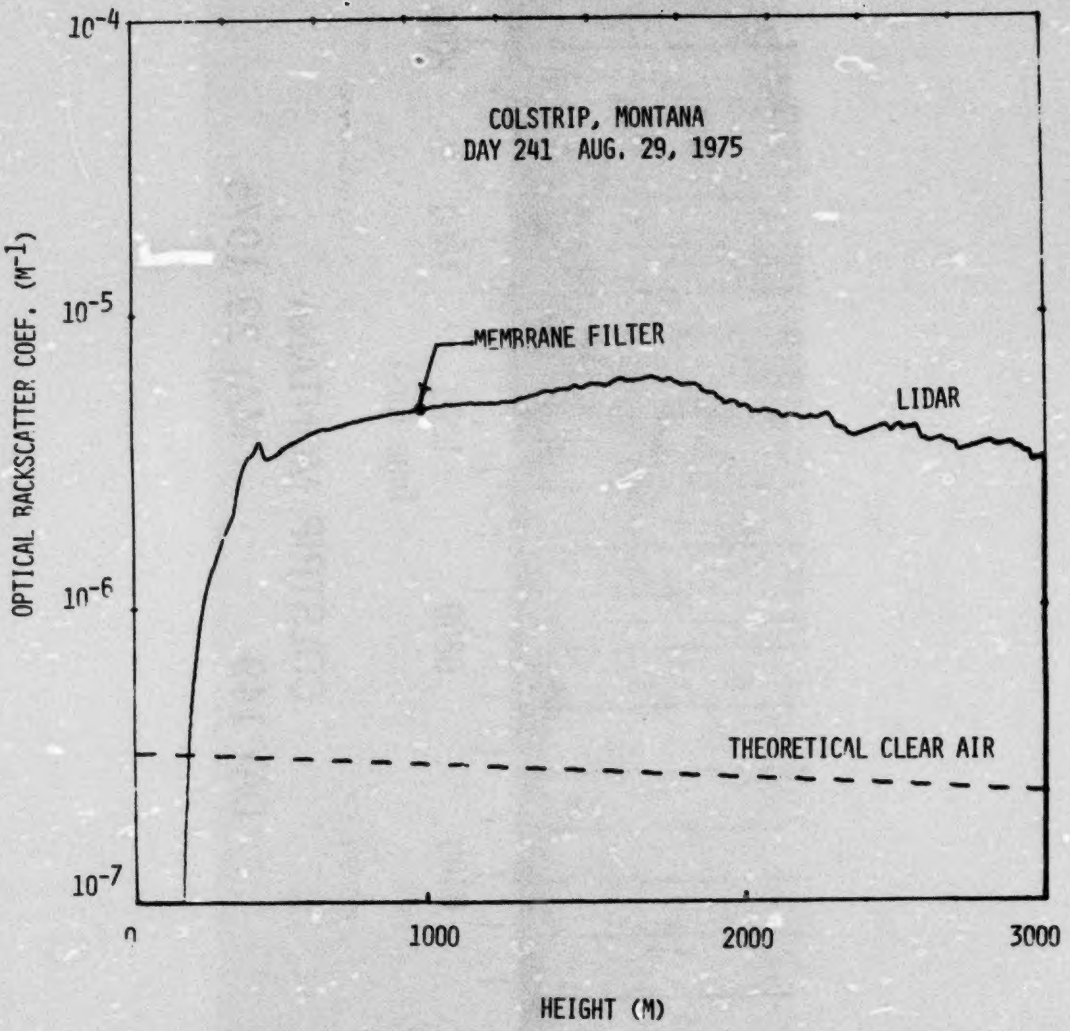

Figure 8.4. Optical backscatter--Fall 1975.

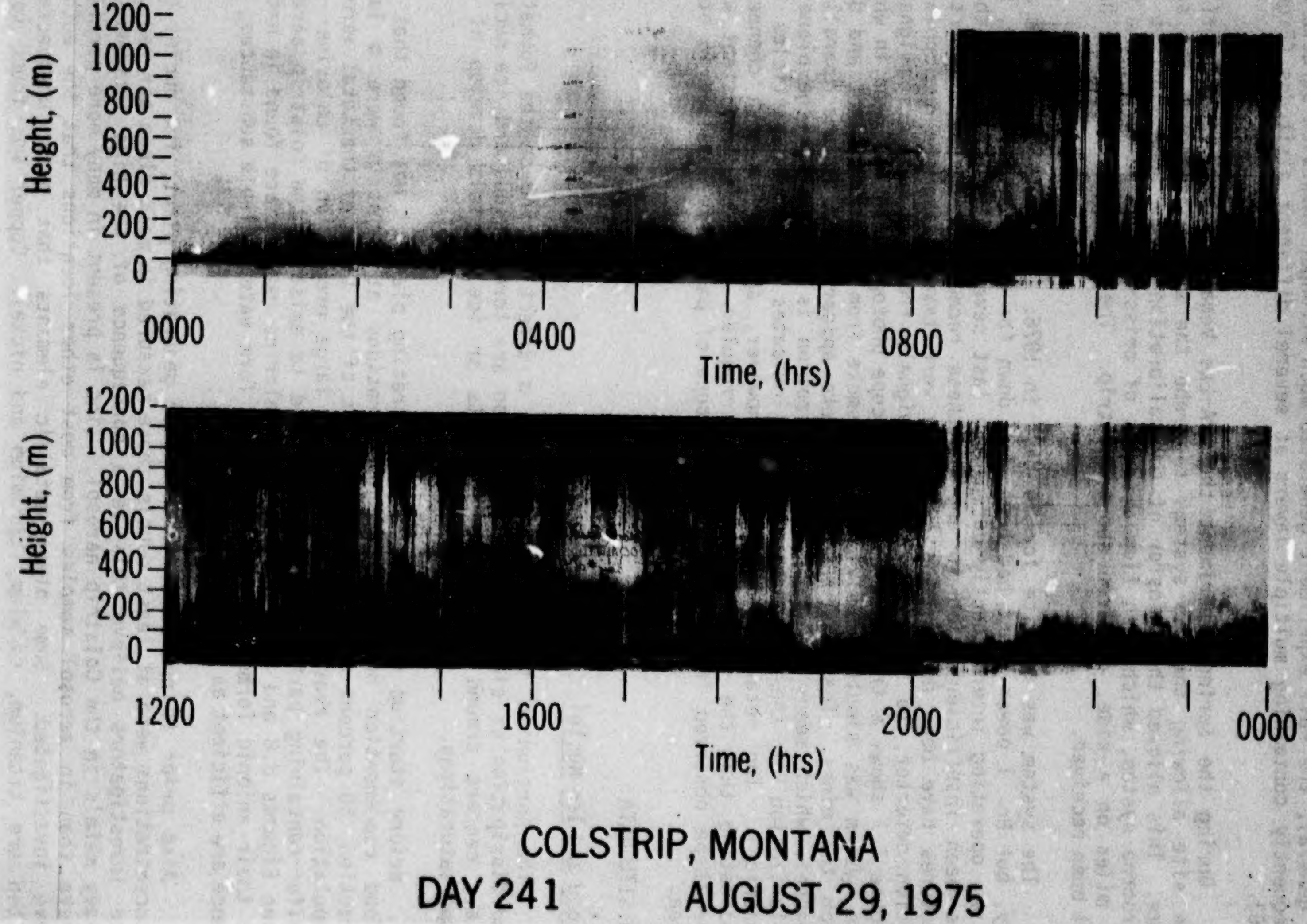

Figure 8.5. Acoustic sounder record--Fall 1975.

In general, the Fall experiment generated much stronger inversion structures, frequently containing multiple layers at several different altitudes (Figure 8.6).

During the Spring experiment the EPA-Las Vegas airborne lidar overflew the site allowing the two systems to probe the same airspace at the same time. This allowed the transfer of the calibration of the NOAA lidar to the airborne system, which then flew a series of criss-crossed patterns in a box 30 miles on a side, centered about Colstrip. The airborne lidar data have not been received.

The system was on site for 14 days in 1976. The weather was warm and dry, but No. 1 power plant had been shut down for maintenance. However, No. 2 was operating intermittently during the last several days. Although there has been insufficient time to analyze these recent data, some interesting features have been noted. The 1976 data were taken using the dual-polarization detector, to separate the stack signals from ordinary dust signals. Figure 8.7 shows a tracing of an oscilloscope photograph of raw data in which the system was simultaneously observing smoke from the power plant and dust from the mines. The return from the smoke appears only in the "parallel" channel, which means its plane of polarization is parallel to the plane of polarization of the laser beam. This indicates the smoke particles are spherical. The return from the dust, however, appears in both channels, indicating that the dust particles are irregular. This is consistent with the shape observed in an electron microscope of particles obtained in other areas.

## IN SITU DATA

### Cloud and Ice Nuclei

The portion of the aerosol that has a direct bearing on the formation and dissipation of clouds and precipitation are termed cloud and ice nuclei, i.e., centers around which water droplets or ice crystals develop at low supersaturations.

Before start-up of the Colstrip generating plant it was found that the cloud condensation nuclei (CCN) concentrations at Colstrip were a large fraction, 50 percent or even more for most of the time, of the total aerosol population. The reason for this is the large proportion of chlorine- and sulfur-containing particles that was found to exist in the Colstrip aerosol (see Figures 8.8 and 8.9). Sulphur and chlorine usually are found in nature in their anionic forms, $SO_4^{=}$ and $Cl^-$, that form water soluble substances, and hence are efficient as CCN.

Also prior to operation of the power generating plant, ice nuclei (IN) concentrations were at least double those measured in most clean air regions. The investigators believe this is a consequence of the high incidence of heavy metals in the Colstrip aerosol. Zinc is present in many more particles there than in aerosol samples from most other locations that the authors have investigated. Some other metallic elements that occur unexpectedly often are titanium, cadmium, chromium and nickel. Copper was found to be

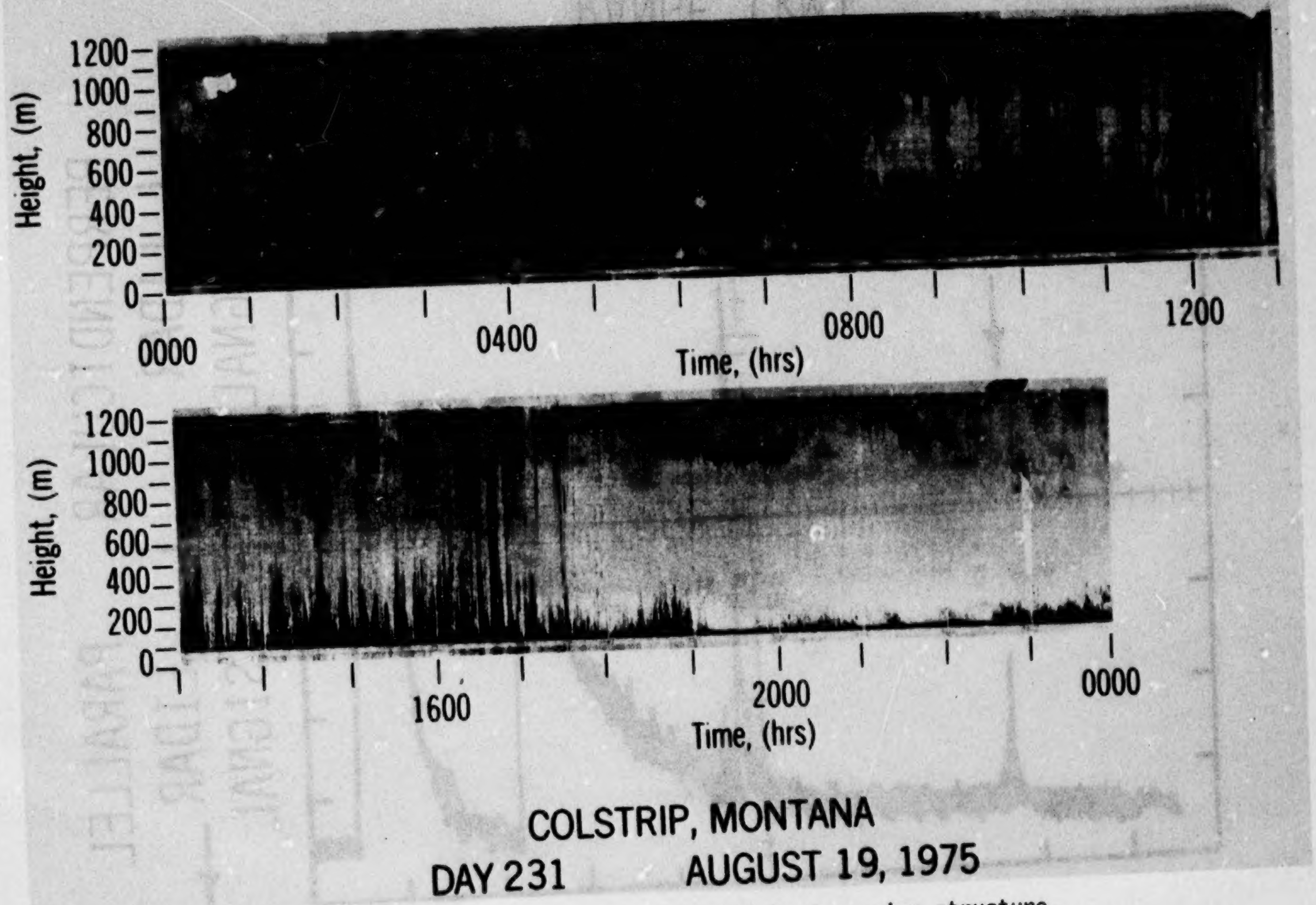

Figure 8.6. Acoustic sounder record showing multiple inversion structure.

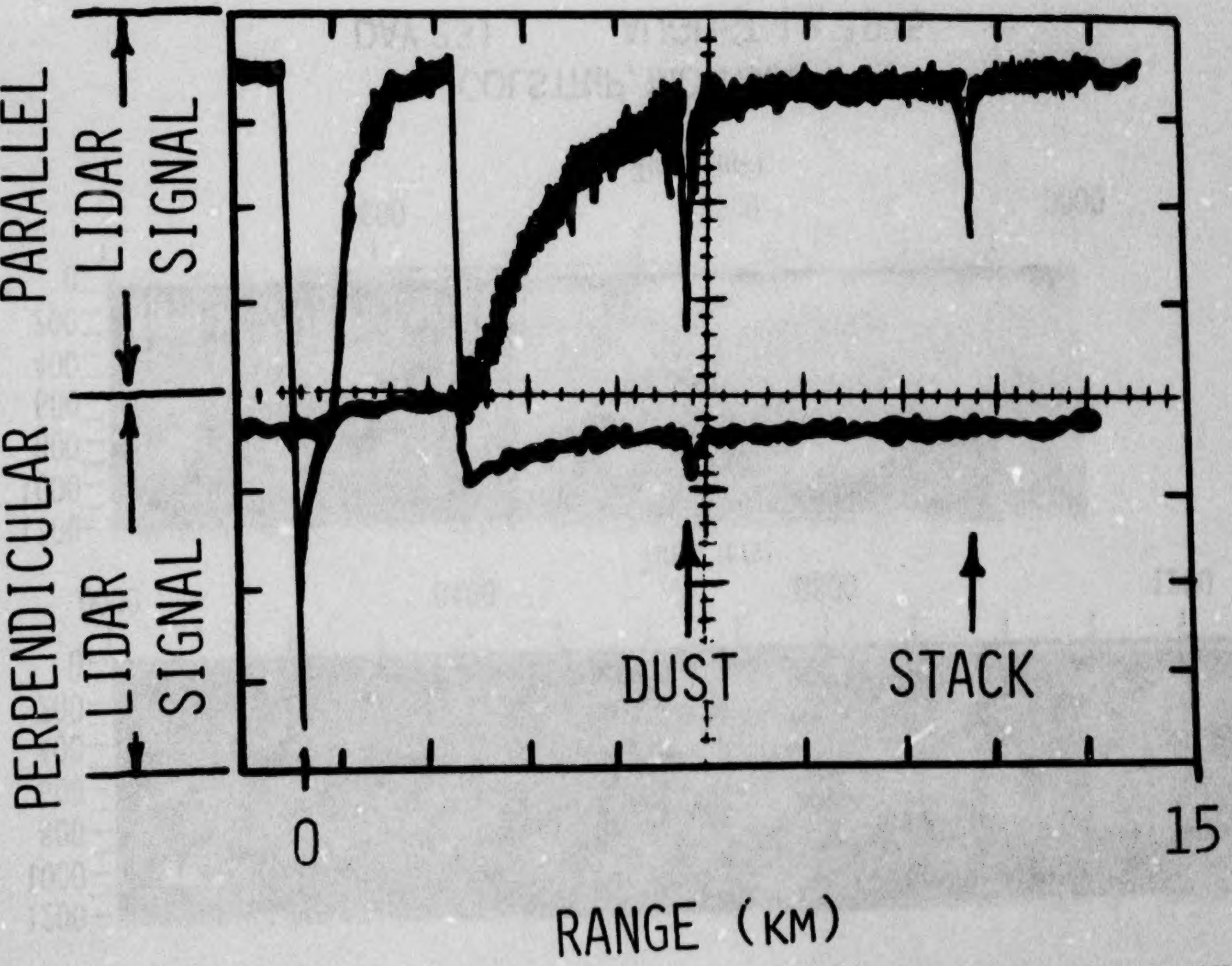

Figure 8.7. Raw lidar return showing difference in depolarization between dust and stack effluent.

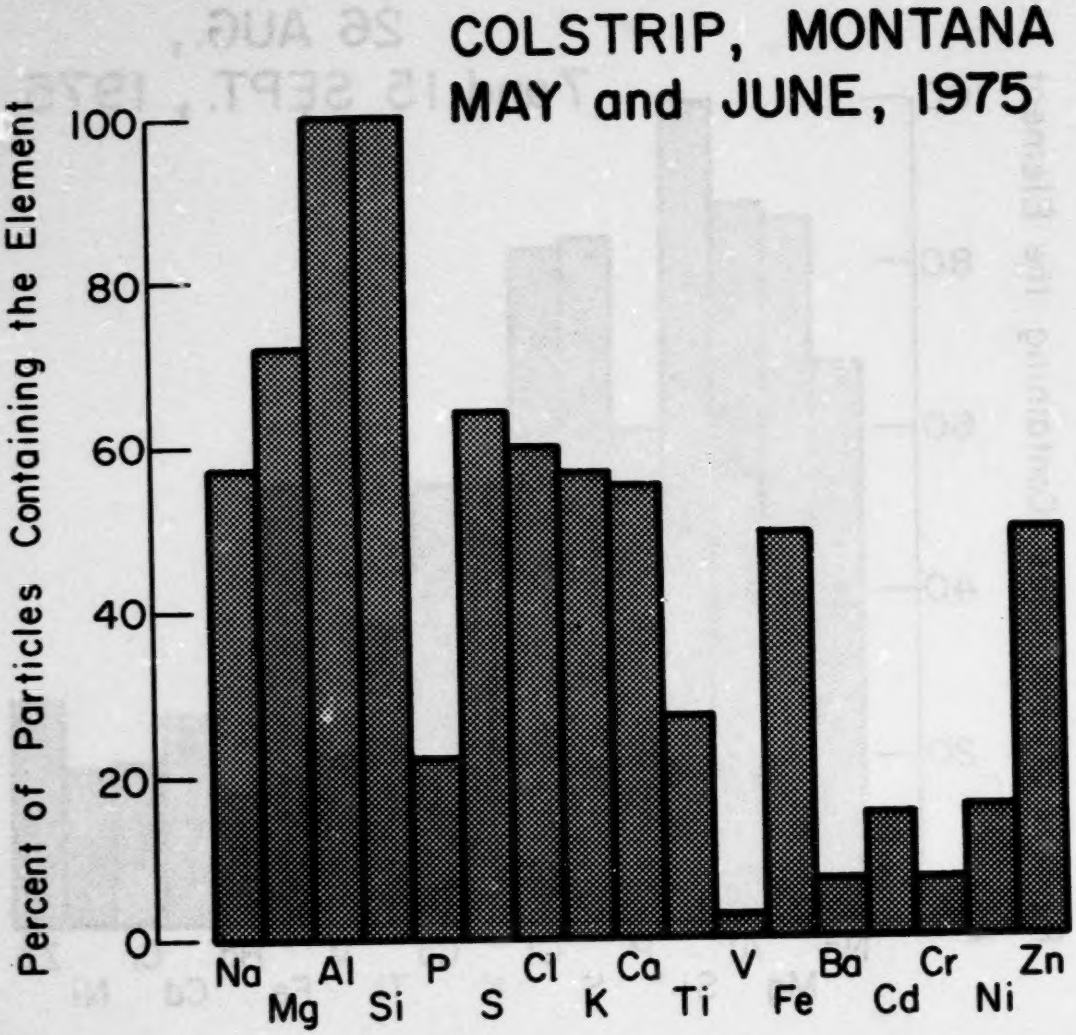

Figure 8.8. Percent of aerosol particles that contain the elements shown, averaged over May and June 1975.

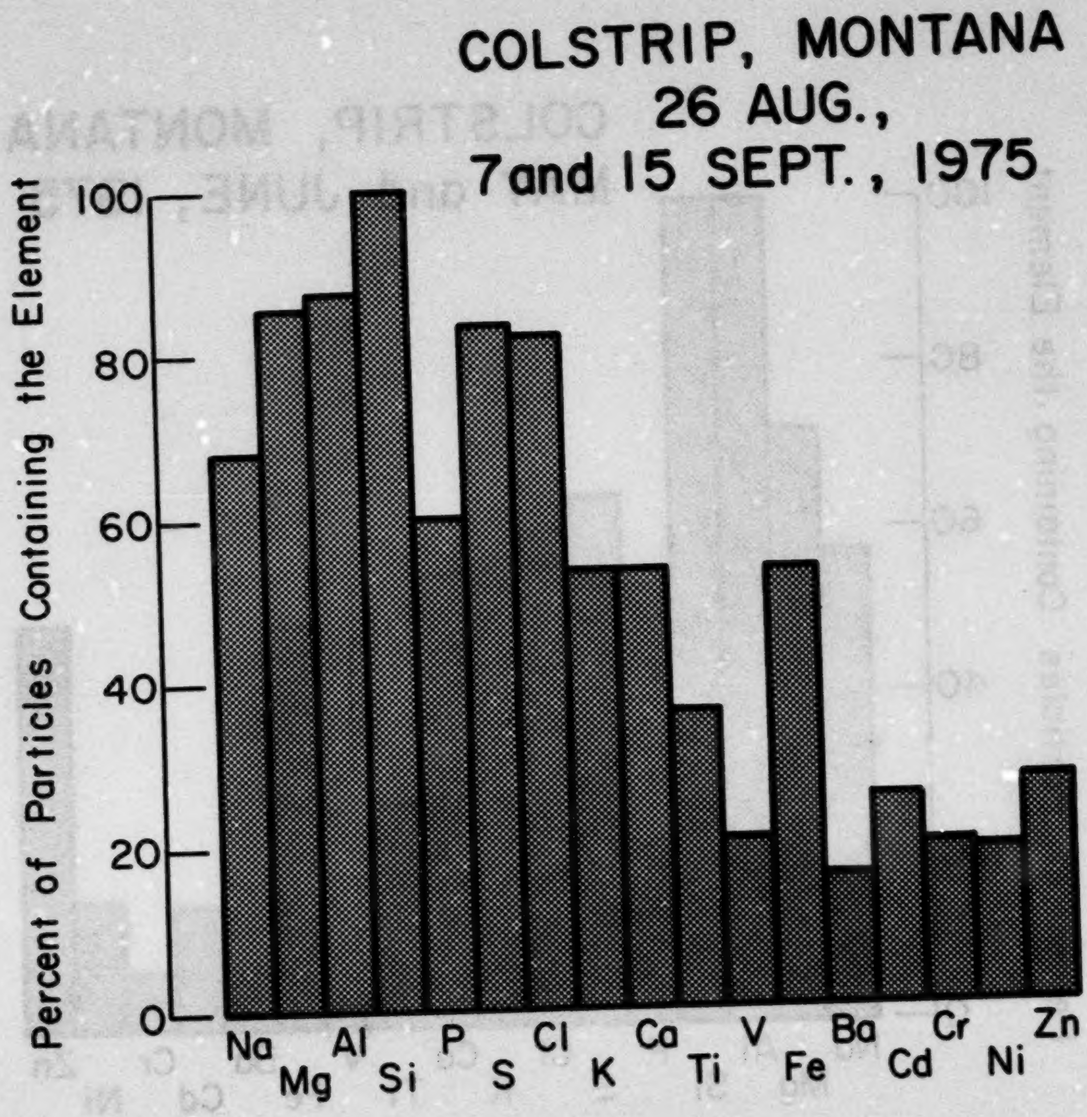

Figure 8.9. Percent of aerosol particles that contain the elements shown, averaged over the three days indicated.

occasionally present. Titanium dioxide and copper sulfide, in particular, have been identified as active ice nucleating materials. In addition, the elements aluminum and silicon have been identified in almost all of the aerosols that were investigated. These elements are widespread in the earth's crust in the form of the common clay mineral, Kaolinite, which is known, in the aerosol form, to be a moderately active ice nucleant.

There exists one other class of atmospheric freezing nuclei. These are biogenically derived and efficient at relatively warm (T > -5°C) temperatures. While the presence of such nuclei is anticipated, there is no means in the investigators' present capability for their detection.

Figures 8.10 and 8.11 are summaries of aerosol properties found during the 1975 field investigations at Colstrip. The light scattering coefficient, $b_{scat}$, is typical of rural western atmospheres; this is discussed further below. The total particle population density (AN, or Aitken nuclei) is also typical of clean atmospheres. The CCN and IN are discussed above.

A cursory look at the record from the airborne operation was done following the May-June 1976 field investigation at Colstrip. From this it is estimated that in the Colstrip power plant plume at short distances from the stacks, the AN and CCN concentrations and $b_{scat}$ are increased by factors of 1.5 to 4.

## AEROSOL OPTICAL PROPERTIES

### Visible-light Optical Effects

Atmospheric aerosols affect the shortwave portion of solar radiation in two ways: (1) They can reduce the amount of solar radiation reaching the ground by scattering a significant portion backward into space, resulting in surface cooling. (2) Absorption of a significant portion of solar radiation, on the other hand, can lead to atmospheric warming. Which of these processes predominates depends on the size and chemical constitution of the aerosol particles.

Results from the analyses of sized and elemental composition (determining the scattering portion of the particle refractive index) were used in a computer program calculating the extinction, scattering and absorption properties of the Colstrip aerosol. From the result it can be concluded that the presently existing aerosol has a net cooling effect at surface albedos less than 0.5, which is the case for the largest portion of the year. Only during the winter months when the ground is snow-covered will the situation be such that a warming effect can be anticipated.

The calculated scattering coefficient was verified with in situ measurements using an integrating nephelometer.

### Infrared Optical Effects

Aerosols absorb IR radiation in the water vapor window, thereby affecting the accuracy of radiometric measurements of, e.g., temperature performed in

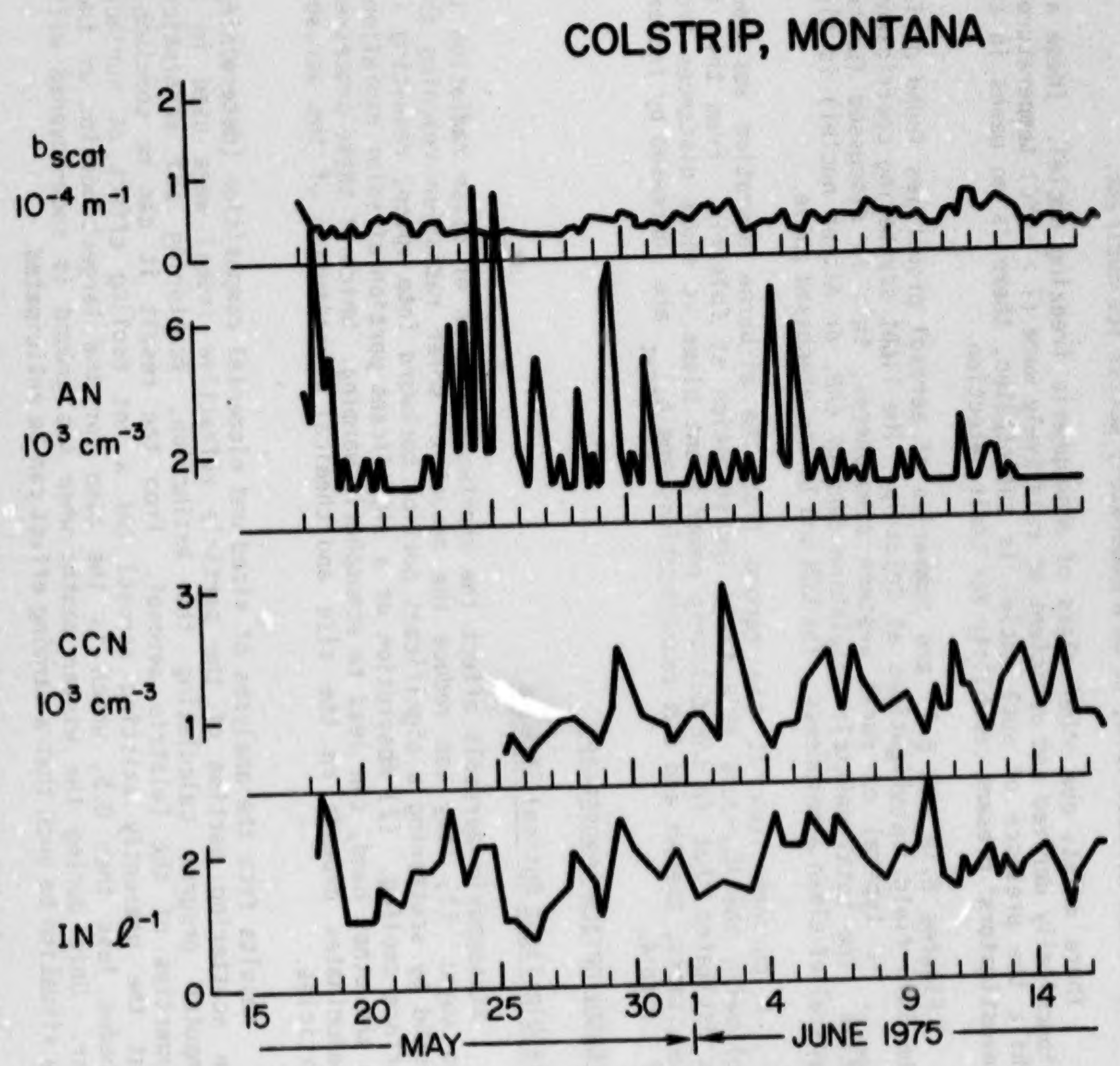

Figure 8.10. Aerosol properties--Spring 1975.

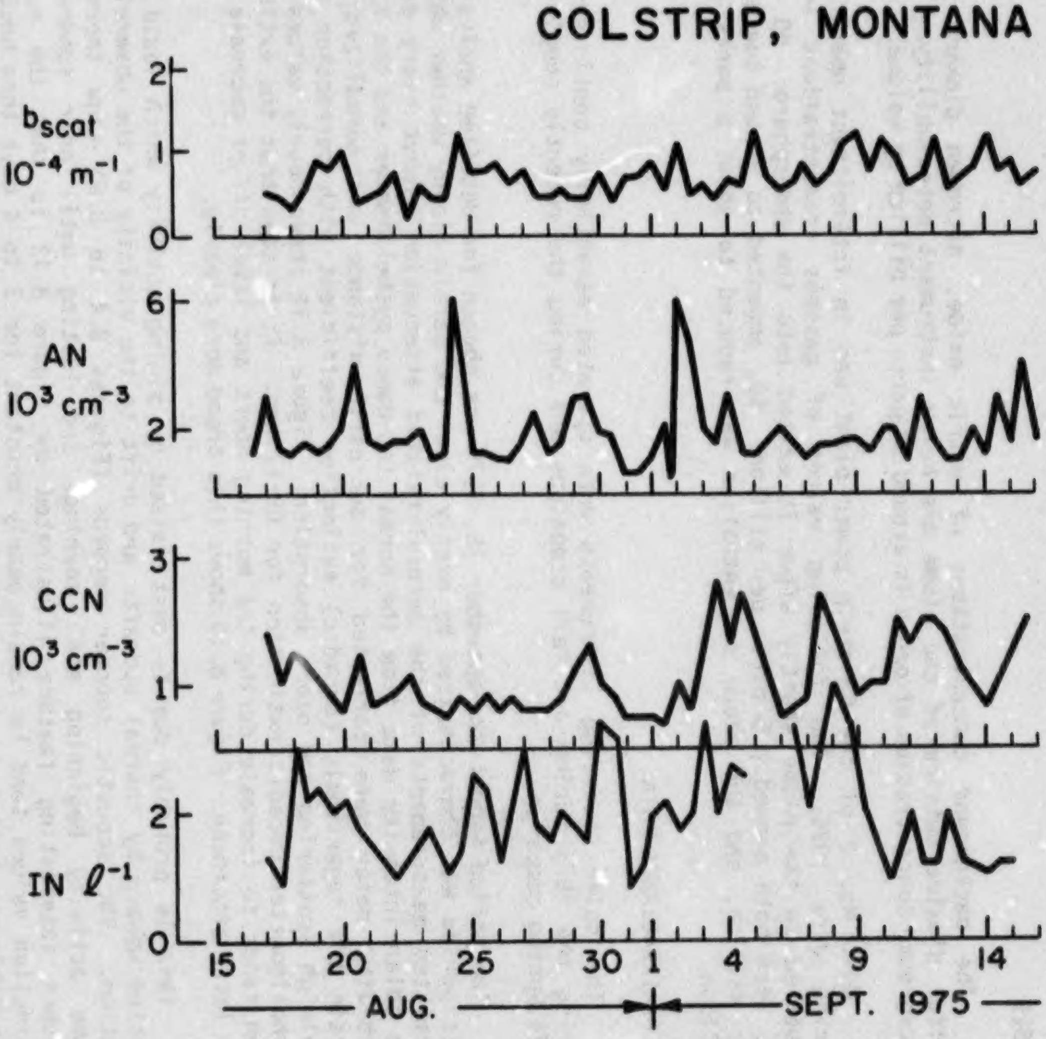

Figure 8.11. Aerosol properties--Fall 1975.

the 8-12 μm band from aircraft and satellites. At the same time they alter the flux of terrestrial radiation into space, a phenomenon that directly affects the earth's heat budget. The magnitude of this effect can be evaluated by measuring with a radiation thermometer in situ at different altitudes the brightness temperature of ground surfaces, which are in thermal equilibrium with the atmosphere. Varying the optical path length by a factor of two, the investigators were unable to detect any effect of the Colstrip aerosol on the earth's equivalent black body temperature.

## GASES

The background concentrations of nitric oxide, nitrogen dioxide and sulfur dioxide outside of the plume are below instrument detectability. The background concentration of ozone is around 30 parts per billion by volume.

Unit No. 2 of the Colstrip power plant was in intermittent operation during June 1976. The following values of gaseous concentrations were observed in the plume shortly after it exited into the atmosphere: NO and $NO_2$ were both around 0.5 parts per million. $SO_2$ amounted to around one part per million, and the ozone concentration was reduced to about 8 parts per billion.

## SOLAR IRRADIANCE DATA

The solar observing instruments were operated essentially continuously during the 1975 Spring and Fall campaigns and during the recently completed 1976 Spring campaign.

The period August 28-September 15, 1975 was chosen for detailed analysis. This period was characterized by mostly clear and partly cloudy weather which permitted measurements of the aerosol-related attenuation almost every day. The solar intensity data from the normal incidence pyrheliometer and the Volz turbidity meter were corrected for sun-earth distance and normalized to obtain the logarithmic (decadic) extinction coefficient with correction for Rayleigh scattering and ozone absorption. Figure 8.12 shows hourly values of pyrhelimometer decadic extinction for 09-17 hrs. It is seen that the extinction tends to increase during the morning hours and level off or decrease in the later afternoon. Figure 8.13 shows this trend more clearly.

This is probably due to dust raised by mining activity which would be carried upward by thermal updrafts and drift to the vicinity of the observing station. The acoustic sounder records (Figures 8.4 to 8.6) show thermal plume activity beginning most mornings and lasting until near sundown. Another interesting feature illustrated by Figure 8.12 is that the noon extinction values tend to remain nearly constant for 3 to 4 days then become changeable or switch to a new level. The cross-hatched areas indicate the stable periods. This behavior is no doubt related to the movement of large scale weather patterns and further study of this feature is planned.

Another way of looking at the variability of atmospheric attenuation is by means of Langley plots (Figure 8.14), which display the logarithm of intensity versus the airmass. The latter is a measure of the amount of air

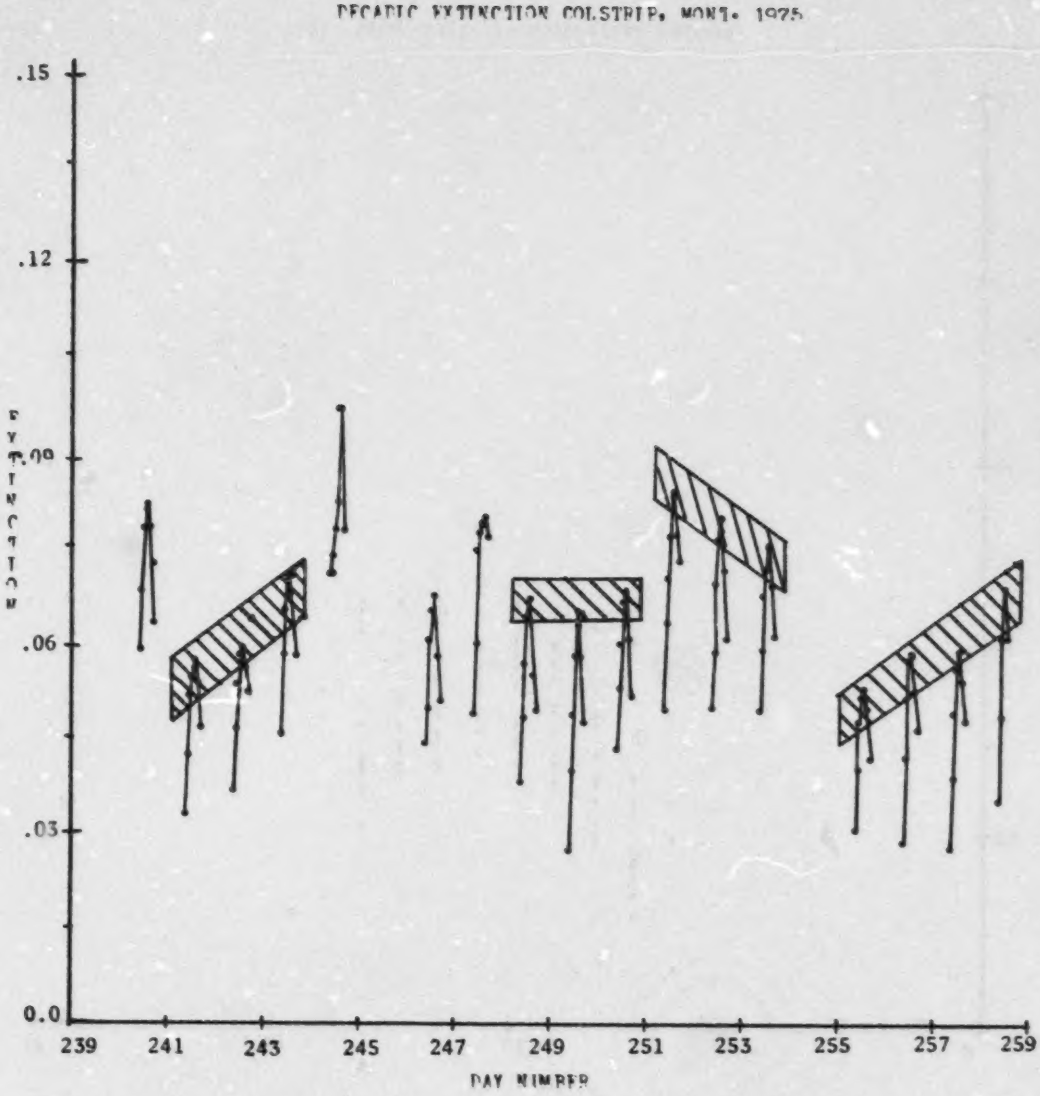

Figure 8.12. Hourly values of pyrheliometer (0.3-2.8 μm) decadic extinction (09-17 hrs) for day numbers 240-258 (Aug. 28-Sept. 15, 1975).

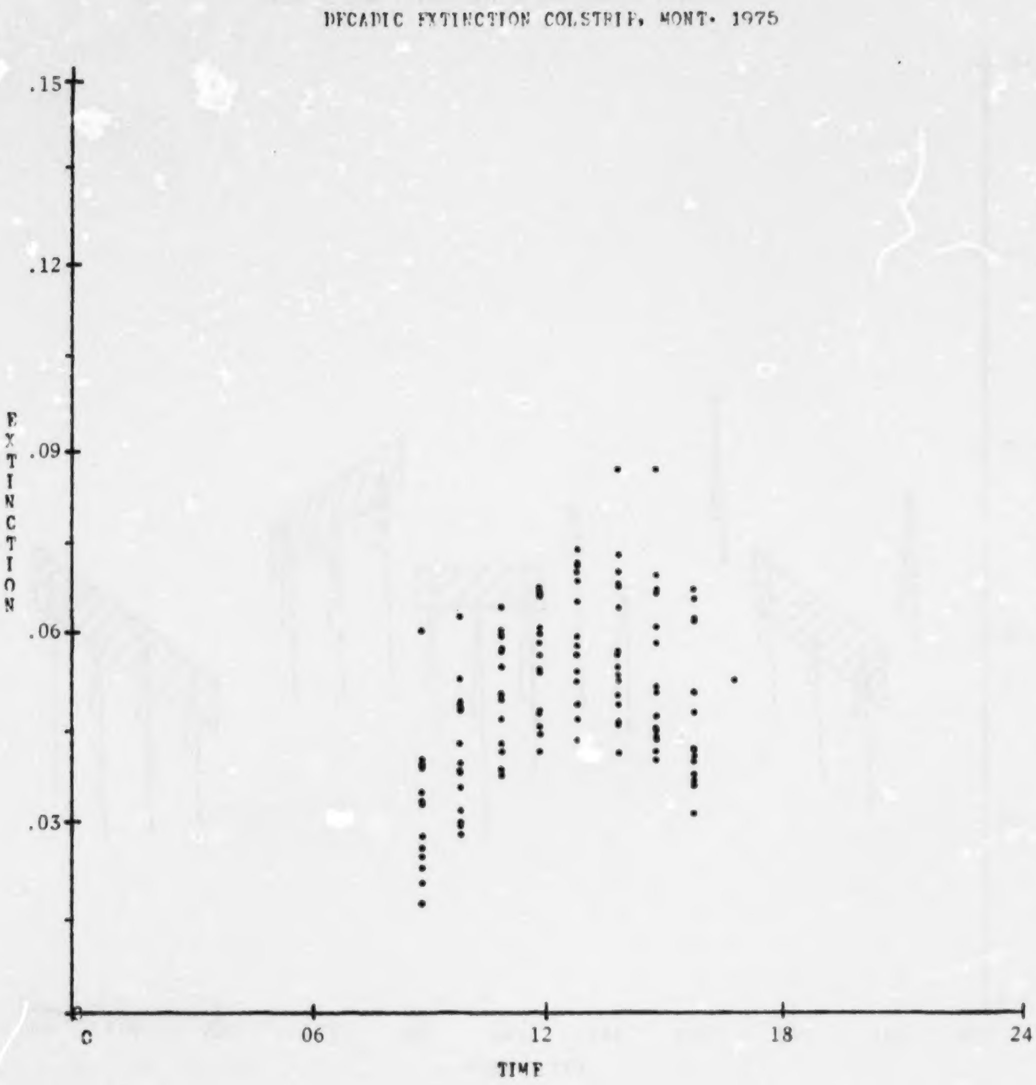

Figure 8.13. Hourly values of pyrheliometer (0.3-2.8 μm) extinction vs time of day for day numbers 240-258, 1975.

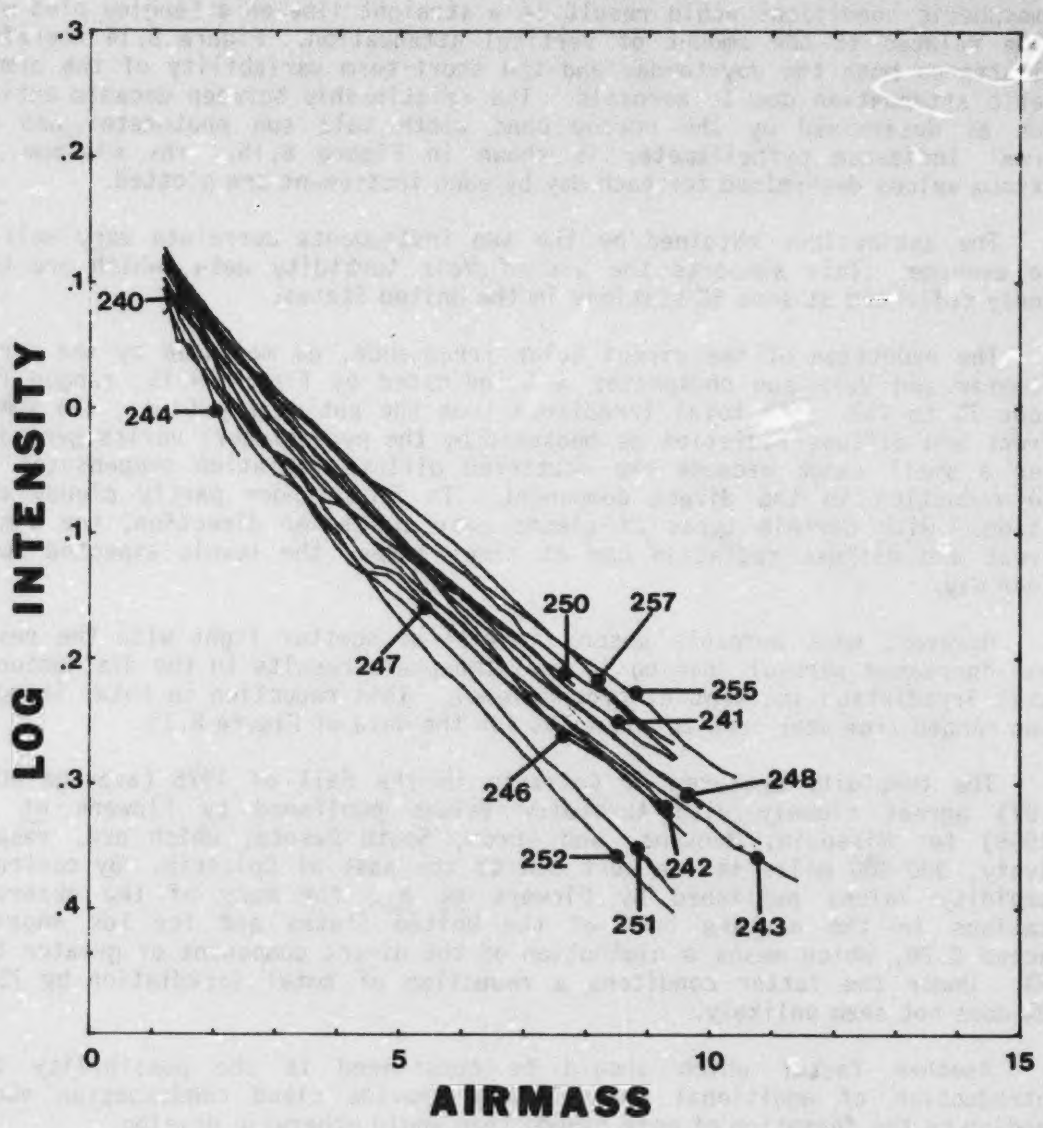

Figure 8.14. Superposed pyrheliometer (3-28 μm) Langley plots for afternoons during period Aug. 28-Sept. 15, 1975 (Day No. 240-258).

through which the solar radiation must pass relative to what it would pass through if the scan were directly overhead. The airmass is approximately equal to sec$\theta$, where $\theta$ is the angle between the vertical and the solar direction, viewed from the observing site. Stable, horizontally uniform atmospheric conditions would result in a straight line on a Langley plot with slope related to the amount of vertical attenuation. Figure 8.14 therefore illustrates both the day-to-day and the short-term variability of the atmospheric attenuation due to aerosols. The relationship between decadic extinction as determined by the narrow band width Volz sun photometer and the normal incidence pyrheliometer is shown in Figure 8.15. The minimum and maximum values determined for each day by each instrument are plotted.

The extinctions obtained by the two instruments correlate very well on the average. This supports the use of Volz turbidity data, which are routinely collected at some 50 stations in the United States.

The reduction of the direct solar irradiance, as measured by the pyrheliometer and Volz sun photometer and indicated by Figure 8.15, ranged from about 7% to 25%. The total irradiance from the entire sky (i.e., the sum of direct and diffuse radiation as measured by the pyranometer) varies generally over a small range because the scattered diffuse radiation compensates for the reduction in the direct component. In fact, under partly cloudy conditions, with certain types of clouds near the solar direction, the sum of direct and diffuse radiation can at times exceed the levels expected for a clear day.

However, most aerosols absorb as well as scatter light with the result that increased aerosol loading in the atmosphere results in the diminution of total irradiation incident at ground level. This reduction in total irradiation ranged from near zero to about 10% for the data of Figure 8.15.

The turbidity measured at Colstrip in the Fall of 1975 (average about 0.07) agrees closely with turbidity values published by Flowers et al. (1969) for Missoula, Montana, and Huron, South Dakota, which are, respectively, 300-400 miles to the west and to the east of Colstrip. By contrast, turbidity values published by Flowers et al. for many of the observing stations in the eastern half of the United States and for Los Angeles, exceed 0.20, which means a diminution of the direct component of greater than 37%. Under the latter conditons a reduction of total irradiation by 15 to 20% does not seem unlikely.

Another factor which should be considered is the possibility that introduction of additional aerosols may provide cloud condensation nuclei leading to the formation of more clouds than would otherwise develop.

## CONCLUSIONS

This section presents a summary of the conclusions at this stage of the observations. The principal conclusions of this study will come only after observations are made while the power plants are burning coal. During 1975 observations there was no plant operation. In Spring 1976, only sporadic operation of Plant No. 2 occurred in a gas-fired mode.

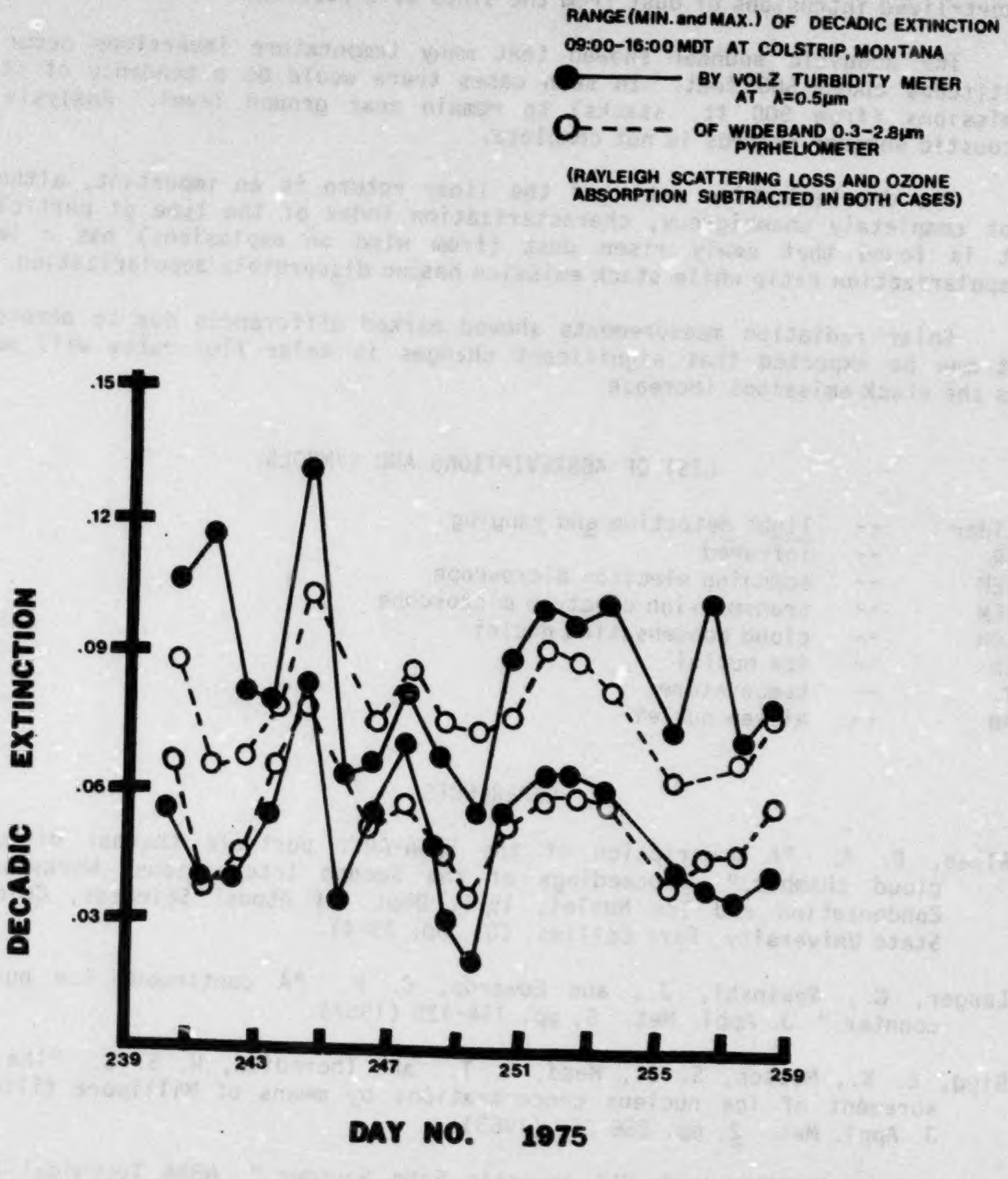

Figure 8.15. Minimum and maximum values of decadic extinction as determined by pyrheliometer (dashed curve) and by Volz sun photometer (solid curve) for day numbers 240-258, 1975.

Observations in 1975 showed a very pristine atmosphere. Total particle counts below 500 per liter were observed. The lidar returns showed very little aerosol content over the whole range of the lidar. Occasional, mild short-lived intrusions of dust from the mines were observed.

The acoustic sounder showed that many temperature inversions occur at altitudes above 500 feet. In such cases there would be a tendency of stack emissions (from 500 ft. stacks) to remain near ground level. Analysis of acoustic sounder records is not complete.

The depolarization ratio of the lidar return is an important, although not completely unambiguous, characterization index of the type of particles. It is found that newly risen dust (from wind on explosions) has a large depolarization ratio while stack emission has no discernible depolarization.

Solar radiation measurements showed marked differences due to aerosols. It may be expected that significant changes in solar flux rates will occur as the stack emissions increase.

## LIST OF ABBREVIATIONS AND SYMBOLS

Lidar -- light detection and ranging
IR -- infrared
SEM -- scanning electron microscope
TEM -- transmission electron microscope
CCN -- cloud condensation nuclei
IN -- ice nuclei
T -- temperature
AN -- Aitken nuclei

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# FIELD AND LABORATORY EXPERIMENTS

## SECTION 9

# ZONAL AIR POLLUTION SYSTEM: DESIGN AND PERFORMANCE

by

J.J. Lee and R.A. Lewis

## INTRODUCTION

A major objective of the Montana Coal-fired Power Plant Project is the generation of a protocol that will improve ability to assess the impact of energy generation on the environment prior to critical land use decisions concerning the initiation of power plant construction. The field experimental component of this project is expected to provide data essential to the construction of such a protocol. In particular, the investigators hope to establish the specificity, thresholds, and temporal interrelations of the effects of sulfur dioxide on well-defined components of a grassland ecosystem. Also, by correlation of results of the field experiments with those from the field studies near Colstrip, Montana, and perhaps elsewhere, they hope to evaluate the site-specificity of these responses. Finally, during a five-year investigation the investigators hope to generate predictive models that will link short-term low threshold effects to those that occur only after prolonged low level exposure.

The experiments are being conducted within two 27-acre grassland exclosures in the Custer National Forest in southeastern Montana. Sulfur dioxide ($SO_2$) fumigation of four one-acre plots within the first exclosure was initiated in May 1975. Fumigation of the second set of four plots within a second exclosure was started in April 1976. Livestock are excluded to protect them from injury and also to protect equipment from damage.

The experiments are designed to test the effects of $SO_2$ upon biomass dynamics (plant, arthropod, and small mammals), plant and animal community structure, insect and fungal diseases of plants, pollination systems, lichens, and upon a number of physiological and biochemical functions. Dominant plants on the study plots are western wheatgrass (Agropyron smithii), prairie junegrass (Koeleria cristata), and Sandberg bluegrass (Poa secunda).

By using a gas delivery system developed for the project (Zonal Air Pollution System--ZAPS), the investigators are able to maintain a selected median concentration of sulfur dioxide on each plot during the entire growing season (circa April through October). Continuous monitoring and control of gas concentrations insure maintenance of the desired levels. Since the plots are ecologically and physiographically similar, observed differences in effects among plots can be attributed to $SO_2$ concentration. Fumigations are planned to continue for at least four growing seasons for the first set of plots, and at least three for the second set.

A prototype system was designed and tested during September 1974. Results indicated the feasibility of this type of control (Lee, Lewis and Body, 1975, 1976b; Lee and Lewis, 1976a). Predictions were made regarding the performance characteristics of the system (see Table 9.4). Analysis of $SO_2$ data for the 1975 season confirmed these predictions (which were based upon modeling solutions and tests of the prototype) and provided further evidence of the adequacy of the system design.

## DESIGN REQUIREMENTS

The ultimate design goal was to provide a system for the unambiguous assessment of the sulfur dioxide impact on otherwise undisturbed grassland. The designers approached this ideal by establishing realistic and appropriate ecological and physical design criteria.

Disturbance of biota and of micro-climates by the system was to be minimized. Effects on incident radiation, prey refuges, ground level obstructions and pathways, temperature, humidity, wind and other features of the micro-habitats were to be kept as small as possible. The area to be stressed had to be large, on the spatial scales of the populations to be sampled, to reduce edge effects and to ensure adequate population and sample sizes. Furthermore, rational application of the system would require that areas chosen for comparisons (i.e., "treatment" and "control" plots) be as nearly uniform as possible in habitat, edaphic, and terrain features.

The logarithms of pollutant concentrations were to follow a normal distribution as occurs in polluted areas (HEW, 1970). The distribution was to be spatially uniform or nearly so, at least on a time average basis, and concentrations were to be controllable for a range of selected averages. Concentrations outside the plots and cross-fumigations among the plots were to be minimized. Finally, cost, maintenance, and operation had to be reasonable.

## THE GAS DELIVERY SYSTEM

The system that was designed and placed in operation for this investigation generally meets the above criteria. Each gas delivery system consists of a network of one inch aluminum pipes (schedule 40, alloy 6061-T6) set parallel to the ground and supported at twenty foot intervals by five foot pipes driven 2 1/2 feet into the ground (Figure 9.1). Release points (1/32 inch horizontal holes) are situated at 10 foot intervals, so that no location within a plot is more than 18 feet from a source. A continuous flow of air through the lines is provided by a helical compressor (Becker model SV 80-1). On all but one of each set of four plots, $SO_2$ is bled into the air stream. Equipment is housed in a heated shed with the temperature kept high enough so that the vapor pressure of the $SO_2$ in the tanks is always above the desired release pressure. Pressure regulators on the tanks and a flow controller (Brooks model 8944) and flowmeter (Brooks model 1110) at the injection point assure the desired flow rate of $SO_2$. Air pressure in the lines (about one pound per square inch, gauge (psig)) varies by less than 5% across the system.

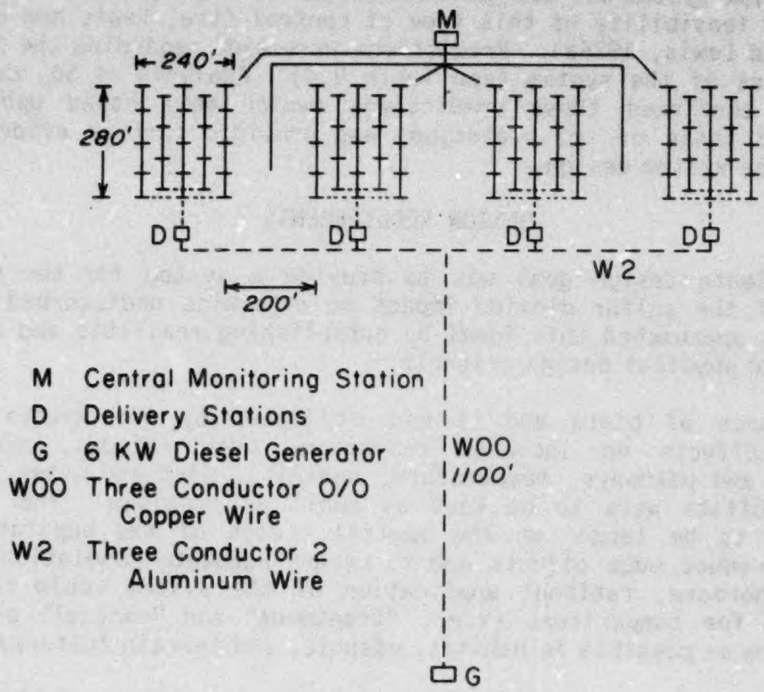
M
240'
280'
D
D
D
D
W2
200'
WOO
1100'
G
M Central Monitoring Station
D Delivery Stations
G 6 KW Diesel Generator
WOO Three Conductor O/O Copper Wire
W2 Three Conductor 2 Aluminum Wire

Figure 9.1. Schematic of the first set of Zonal Air Pollution Systems showing the individual delivery systems and the common monitoring and electrical systems. CONTROL, LOW, MEDIUM and HIGH indicate relative $SO_2$ exposures on the experimental plots.

The only ground level obstructions within the plots are the supports. These should cause minimum interference with animal movements, while the pipe network should negligibly influence the micro-climate. The relatively small size of the plots precludes the study of large animals, although insects, other arthropods and small rodents are included. The contiguity of plots allowed selection for nearly uniform habitat conditions.

The four plots of each set are located along a line, with intervening buffer zones to minimize interference between plots (Figure 9.2). To achieve desired ambient concentrations during 1975 (Table 9.4), three of four plots of the first set of plots received $SO_2$ at the constant rates of 1.5, 3.5, and 7.0 standard cubic feet per hour (SCFH) per plot. This implies that the $SO_2$ concentration in the delivery lines is approximately 1/2 - 1% (volume) for the largest $SO_2$ rate; it is correspondingly lower for plots with lower $SO_2$ flow

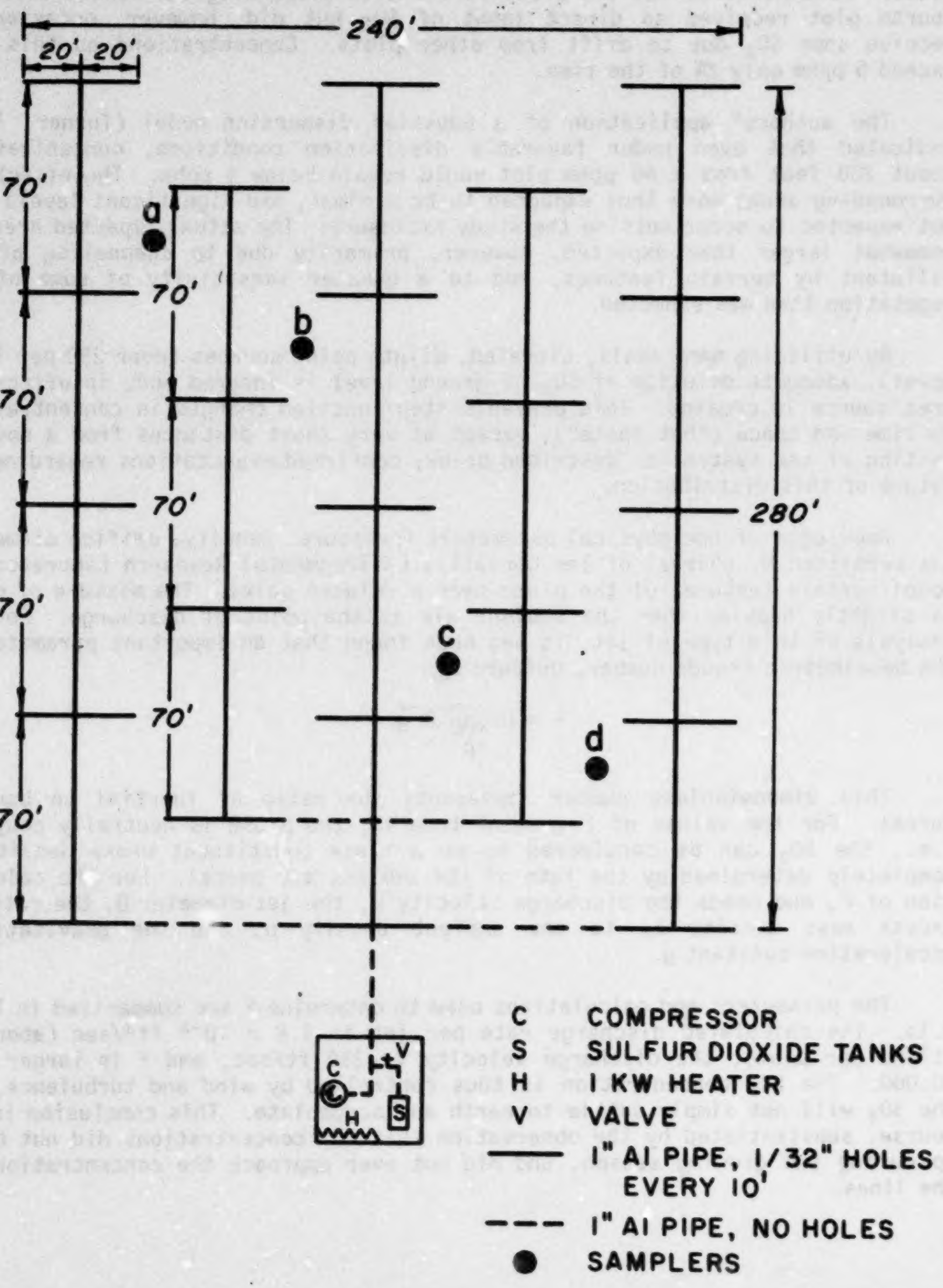
240'
20'
20'
70'
70'
70'
70'
70'
70'
280'
a
b
c
d
C
V
H
S
C COMPRESSOR
S SULFUR DIOXIDE TANKS
H 1 KW HEATER
V VALVE
1" Al PIPE, 1/32" HOLES EVERY 10'
1" Al PIPE, NO HOLES
SAMPLERS

Figure 9.2. Schematic of a single Zonal Air Pollution treatment plot.

rates. Concentrations on the plots varied with meteorological conditions. The fourth plot received no direct input of $SO_2$ but did, however, occasionally receive some $SO_2$ due to drift from other plots. Concentrations on this plot exceed 5 pphm only 2% of the time.

The authors' application of a Gaussian dispersion model (Turner, 1969) indicated that even under favorable dissipation conditions, concentrations about 200 feet from a 40 pphm plot would remain below 5 pphm. The effects on surrounding areas were thus expected to be minimal, and significant levels were not expected to occur outside the study exclosure. The actual impacted area was somewhat larger than expected, however, primarily due to channeling of the pollutant by terrain features, and to a greater sensitivity of some of the vegetation than was expected.

By utilizing many small, elevated, dilute point sources (over 250 per 1 1/4 acres), adequate dilution of $SO_2$ at ground level is insured and, in effect, an area source is created. This prevents step-function changes in concentrations in time and space ("hot spots"), except at very short distances from a source. Testing of the system, as described below, confirmed expectations regarding the nature of this distribution.

Knowledge of the physical parameters (pressure, density, orifice diameter) has permitted M. Shirazi of The Corvallis Environmental Research Laboratory to model certain features of the plume near a release point. The mixture of gases is slightly heavier than the ambient air at the point of discharge. For the analysis of this type of jet, it has been found that an important parameter is the densimetric Froude number, defined by

$$F = U/\sqrt{\frac{\Delta\rho}{\rho} D\ g}$$

This dimensionless number represents the ratio of inertial to bouyant forces. For the values of F greater than 40, the plume is neutrally bouyant; i.e., the $SO_2$ can be considered to be a trace constituent whose density is completely determined by the fate of its ambient air parcel. For the calculation of F, one needs the discharge velocity U, the jet diameter D, the ratio of excess mass density $\Delta\rho$ to the ambient density $\rho$, and the gravitational acceleration constant g.

The parameters and calculations used to determine F are summarized in Table 9.1a. The calculated discharge rate per jet is $1.8 \times 10^{-3}$ $ft^3/sec$ (about 27 $ft^3/hr$ per plot), the discharge velocity is 330 ft/sec, and F is larger than 10,000. The $SO_2$ concentration is thus controlled by wind and turbulence, and the $SO_2$ will not simply settle to earth and accumulate. This conclusion is, of course, substantiated by the observation that $SO_2$ concentrations did not build up during the growing season, and did not ever approach the concentrations in the lines.

## TABLE 9.1a. SUMMARY OF PARAMETERS AND CALCULATIONS USED TO ESTIMATE DENSIMETRIC FROUDE NUMBER.

---

Mass Flow Through Jet:

$$W = 223.8\ \frac{APo}{R}\ \left(\frac{C_p}{To}\ \left[\left(\frac{P}{Po}\right)^{2/k} - \left(\frac{P}{Po}\right)^{(k+1)/k}\right]\right)^{1/2} \text{ lb/sec} = 1.4 \times 10^{-4} \text{ lbs/sec}$$

Where A = area of 1/32 in discharge jet, $ft^2$

Po = pressure in line, 15.7 psi

P = pressure of ambient air, 14.7 psi

To = absolute temperature, 540° R

Cp = specific heat of air, constant p, .24 BTU/lb - °R

k = Cp/Cv where Cv is specific heat at constant volume, k = 1.4

R = universal gas constant divided by molecular wt. of air, 53.3 ft-lb/lb - °R

Volume Flow Through Jet:

$$V = \frac{WRTo}{Po} = 1.8 \times 10^{-3} \text{ ft}^3\text{/sec}$$

Discharge Velocity: U = V/A = 332 ft/sec

Excess Density of 1% (volume) Gas Mixture:

$$\frac{\rho_{mix}}{\rho_{ambient}} = \frac{(\text{molecular wt})_{mix}}{(\text{molecular wt})_{ambient}} = \frac{.99 \times 28.97 + .01 \times 64.06}{28.97} = 1.012$$

$$\frac{Wp}{\rho} = .012$$

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The analyses of this type of problem indicate that jet dilution in still air can be estimated by

$$\theta/\theta_o = .32\ X/D$$

where θ is the total entrained ambient air within the plume at a distance X/D jet diameters away from the point of discharge. Examples of dilution for several distances are given in Table 9.1b. The actual observed dilutions (Table 9.8) were at least 15 times greater than those predicted by this rough model.

TABLE 9.1b. PREDICTED DILUTION FACTORS AT VARIOUS DISTANCES FROM DISCHARGE POINT.

| Distance (ft) | Dilution Factor |
|---|---|
| 0.5 | 62 |
| 1.0 | 123 |
| 3.0 | 369 |
| 5.0 | 615 |

## SYSTEM PERFORMANCE

A full size prototype of the system was constructed and employed to test the feasibility of this experimental approach to the field during October 1974. Results of these tests (Lee, Lewis and Body, 1975, 1976b; Lee and Lewis, 1976a) demonstrated that concentrations across a plot are adequately controlled at the low levels desired, but also suggested an improved design to minimize spatial variation of concentrations. This new design (Figure 9.2) has been utilized in all experimental gasing to date. Analysis of data for one entire growing season indicates that the conclusions regarding the prototype were correct, and that spatial variability is minimized by the new design.

$SO_2$ concentrations on the plots were monitored by recording the output from a Meloy model SA160 sulfur analyzer operating in a logarithmic mode. Samples were drawn through Teflon tubing by a time-share device (Adgo Co.) so that each sample location was monitored at the rate of approximately 8 minutes per hour.

Data on $SO_2$ concentrations were obtained one foot above ground level at central location c (Figure 9.2) for each plot for the entire 1975 growing season. Locations a and b (Figure 9.2) were also monitored for prolonged periods for various plots. Location d was sampled occasionally; these data have not yet been fully analyzed. In addition, specialized, short-term tests of $SO_2$ distribution have been performed. These have been analyzed in terms of temporal and spatial variations of $SO_2$. Comprehensive characterization of horizontal and vertical distributions will continue during 1977.

## TEMPORAL VARIATION OF $SO_2$

The frequency distributions of 8-minute medians (standard location c) are shown in Figure 9.3, while Figures 9.4 and 9.5 show the frequency distributions for 1-hour and 3-hour averages obtained from the 8-minute medians by interpolation and averaging. All the distributions are approximately log-normal, typical of pollutant frequency distributions (HEW, 1970; Larsen, 1969). Such distributions are characterized by the geometric mean (GM) and standard geometric deviation (SGD). These parameters summarize the temporal frequency of occurrence of concentrations, and thus represent a measure of dose. Typical values for actual pollutant distributions are given in Table 9.2. Any area with similar GM and SGD can be said to have received a similar dose.

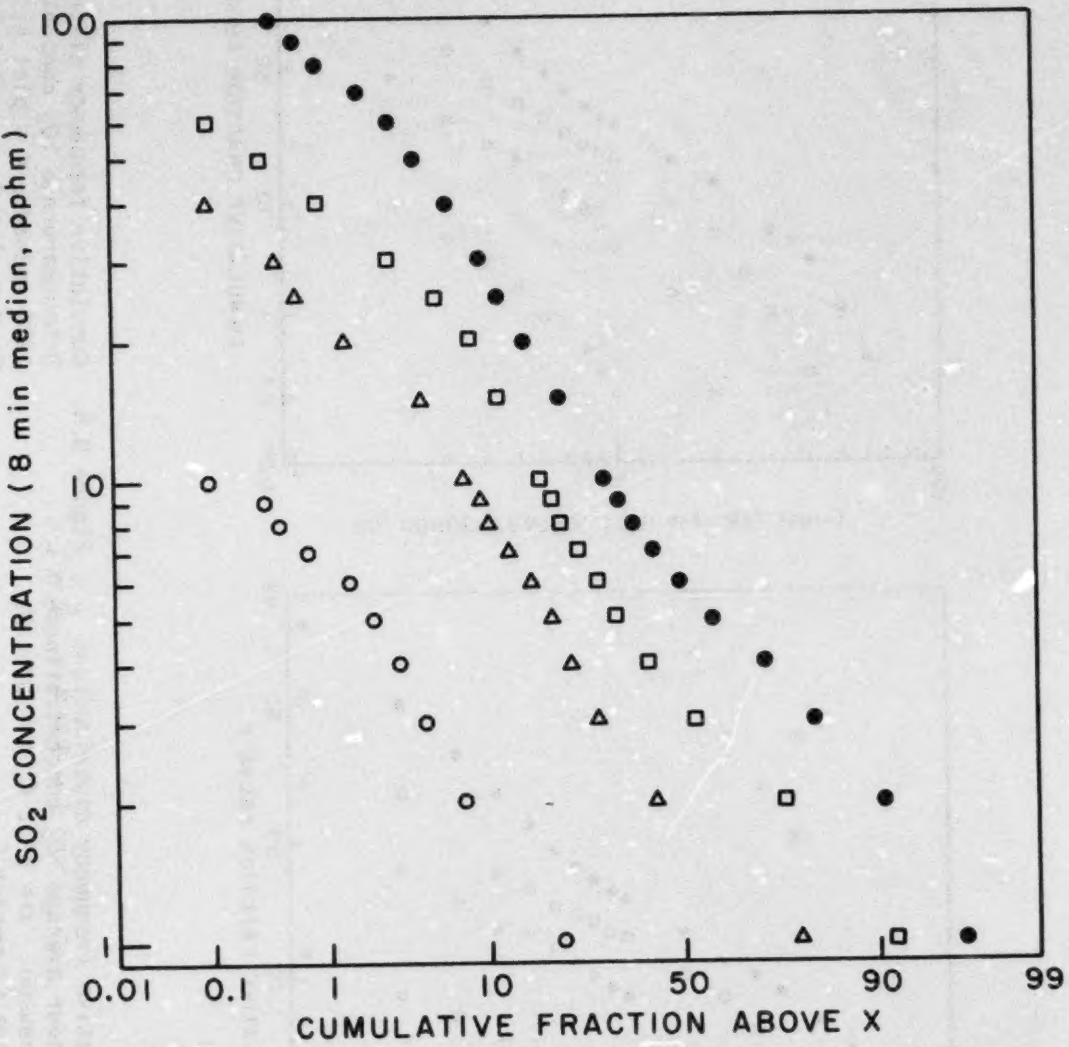

Figure 9.3. Cumulative frequency distributions of 8-min median $SO_2$ concentrations, 1975 season. ○ = plot A; Δ = plot B; □ = plot C; ● = plot D.

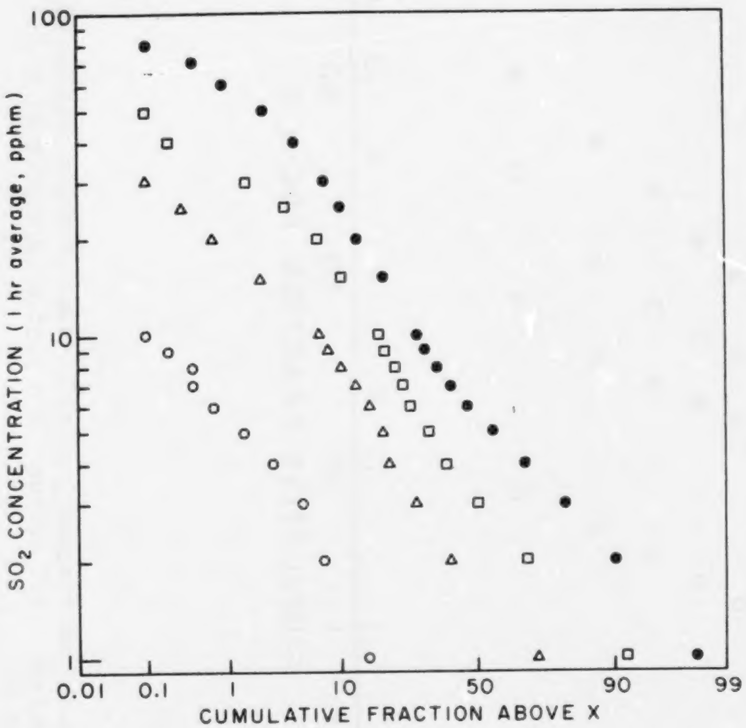

Figure 9.4. Cumulative frequency distributions of 1-hour average $SO_2$ concentrations, 1975 season. ○ = plot A; Δ = plot B; □ = plot C; ● = plot D.

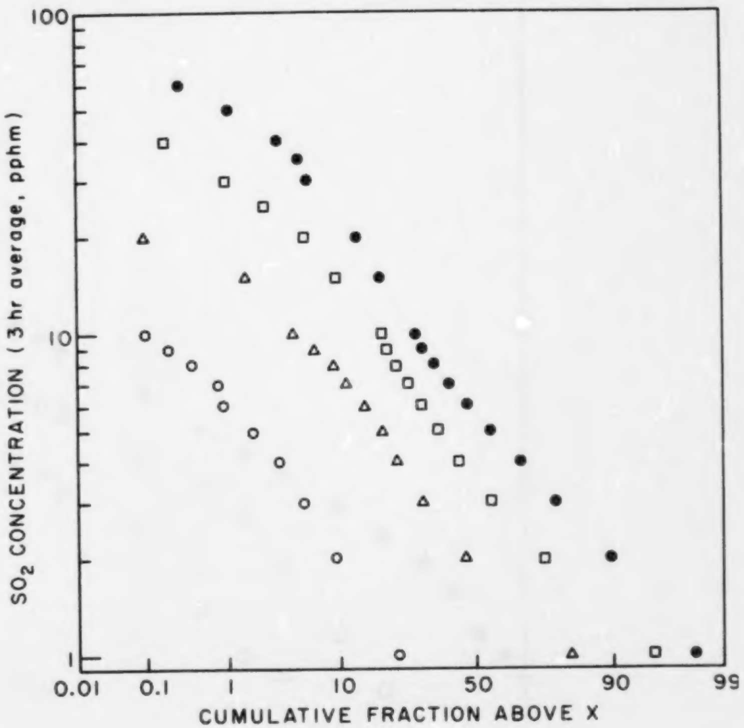

Figure 9.5. Cumulative frequency distributions of 3-hour average $SO_2$ concentrations, 1975 season. ○ = plot A; Δ = plot B; □ = plot C; ● = plot D.

TABLE 9.2. GEOMETRIC MEANS (GM, pphm) AND STANDARD GEOMETRIC DEVIATIONS (SGD) FOR SEVERAL URBAN AREAS. Five Minute Averages 1962-67 (source: 7)

| City | GM | SGD |
|---|---|---|
| Chicago | 10.4 | 2.2 |
| Cincinnati | 1.6 | 2.9 |
| Denver | 1.3 | 2.1 |
| Los Angeles | 1.3 | 2.3 |
| Philadelphia | 5.5 | 2.4 |
| St. Louis | 2.8 | 2.8 |
| San Francisco | 0.5 | 2.9 |
| Washington | 3.9 | 2.2 |

The values of these parameters for the four plots are given in Table 9.3a for the entire growing season, and in Table 9.3b for individual months. The lower concentrations in October were probably due to frequent rain.

Due to the logarithmic mode of the $SO_2$ monitor, all readings of zero on the strip chart are recorded as concentrations of .9 pphm. The minimum recordable GM is thus also .9 pphm. This effect was only important for plot A, which was below .9 pphm more than 75% of the time.

In models of pollutant dispersion source strength appears as a normalization constant, with the patterns of distribution determined by meteorological conditions (Turner, 1969). The resultant low variability of SGD over a range of GM's is evident for the urban areas summarized in Table 9.2 and for the experimental plots in Table 9.3. The only dissimilar SGD is for the plot which did not receive direct $SO_2$ input. The similarity of the SGD's in Tables 9.2 and 9.3 indicates that the fine-scale variability of $SO_2$ concentrations throughout the study plots is realistic and reproducible.

Further evidence of the similarity of variability among study plots is given in Figure 9.6. In this graph the $SO_2$ concentrations have all been normalized to have the same GM as the highest concentation plot, plot D. This was done by multiplying the $SO_2$ concentrations for plot "i" by the ratio GM (plot D): GM (plot "i"). The coincidence of the resulting curves for plots receiving direct $SO_2$ input shows that they differ in "amount" of $SO_2$ (GM) but not in $SO_2$ "variability" (SGD). The curve for the fourth plot is affected by the fact that the true GM is lower than the measured GM. The similarily of the shape of this curve to the other curves is obvious in Figure 9.6.

The authors predicted $SO_2$ distribution parameters and peaks prior to the introduction of $SO_2$ (Lee, Lewis and Body, 1976b). These are compared to the observed values in Table 9.4. The seasonal 3-hour peaks are the most important values from the standpoint of the present Federal Secondary Standard for $SO_2$ (Table 9.5). The differences between medians and geometric means are measures of deviation from true log-normality.

TABLE 9.3a. PARAMETERS OF $SO_2$ FREQUENCY DISTRIBUTIONS FOR 1975 GROWING SEASON.

| Plot | For 8-min Medians |  | For 1-hr Averages |  | For 3-hr Averages |  |
|---|---|---|---|---|---|---|
|  | GM | SGD | GM | SGD | GM | SGD |
| A | 1.0 | 1.5 | 1.0 | 1.5 | 1.0 | 1.6 |
| B | 2.2 | 2.4 | 2.1 | 2.4 | 2.2 | 2.2 |
| C | 3.8 | 2.7 | 3.6 | 2.7 | 3.9 | 2.5 |
| D | 6.8 | 2.7 | 6.4 | 2.6 | 6.2 | 2.6 |

GM: Geometric mean, pphm (minimum recordable is .9 pphm).
SGD: Standard geometric deviation.

TABLE 9.3b. GEOMETRIC MEANS AND STANDARD DEVIATIONS OF 8-MIN. MEDIAN $SO_2$ CONCENTRATIONS ON A MONTHLY BASIS.

| Month | Plot | GM (pphm) | SGD |
|---|---|---|---|
| June | A | 1.18 | 1.50 |
|  | B | 2.54 | 2.05 |
|  | C | 4.40 | 2.40 |
|  | D | 7.52 | 2.75 |
| July | A | 0.96 | 1.38 |
|  | B | 2.43 | 2.29 |
|  | C | 4.47 | 2.49 |
|  | D | 7.59 | 2.86 |
| August | A | 1.02 | 1.62 |
|  | B | 2.31 | 2.44 |
|  | C | 3.54 | 2.56 |
|  | D | 6.38 | 2.52 |
| September | A | 1.02 | 1.59 |
|  | B | 2.32 | 2.61 |
|  | C | 4.42 | 2.89 |
|  | D | 8.37 | 2.69 |
| October | A | 0.97 | 1.45 |
|  | B | 1.43 | 2.17 |
|  | C | 2.32 | 2.63 |
|  | D | 4.02 | 2.46 |

TABLE 9.4. COMPARISON OF PREDICTED[1] AND OBSERVED $SO_2$ PARAMETERS, 8-MIN. AVERAGING TIME, 1975 GROWING SEASON.

| Plot | Geometric Mean (pphm) | | Median (pphm) | | SGD | | Seasonal 3-hr. Peak (pphm) | |
|---|---|---|---|---|---|---|---|---|
| | Predicted | Observed | Predicted | Observed | Predicted | Observed | Predicted | Observed |
| A | 0.0 | 1.0 | --- | --- | --- | 1.5 | 0 | 11 |
| B | 2.0 | 2.2 | 2.0 | 1.7 | 2.1 | 2.4 | 15-20 | 20 |
| C | 5.0 | 3.8 | 5.0 | 3.4 | 2.1 | 2.7 | 40-50 | 47 |
| D | 10.0 | 6.8 | 10.0 | 5.8 | 2.1 | 2.7 | 100-200 | 69 |

[1] Predicted by Lee, Lewis, and Body, 1975.

TABLE 9.5. MONTANA AND FEDERAL STANDARDS, pphm.

| | 1-hr. | 3-hr. | 24-hr. |
|---|---|---|---|
| Montana | 25[a] | | 10[b] |
| Federal Primary | | | 14[c] |
| Federal Secondary | | 50[c] | |

[a] not to be exceeded more than once per four days (approx. 1%)

[b] not to be exceeded more than 1% of time

[c] not to be exceeded more than once per year

TABLE 9.6. PEAKS OBSERVED DURING 1975 SEASON, VARIOUS AVERAGING TIMES, pphm.

| Plot | 8-min | 1-hr | 3-hr | 16-hr |
|---|---|---|---|---|
| A | 14 | 11 | 11 | 3 |
| B | 44 | 32 | 20 | 13 |
| C | 71 | 54 | 47 | 24 |
| D | 140 | 89 | 69 | 33 |

TABLE 9.7. PERCENT OF TIME MONTANA (MT) AND FEDERAL (FED) STANDARD LEVELS WERE EXCEEDED.

| Plot | MT 1-hr | MT 24-hr | Fed 3-hr | Fed 24-hr |
|---|---|---|---|---|
| A | 0 | 0 | 0 | 0 |
| B | 0.3[a] | 1.2 | 0 | 0 |
| C | 3.8 | 27.5 | 0 | 10.0 |
| D | 10.2 | 59.3 | 1.1 | 34.6 |

[a] Not frequent enough to constitute a violation of standard

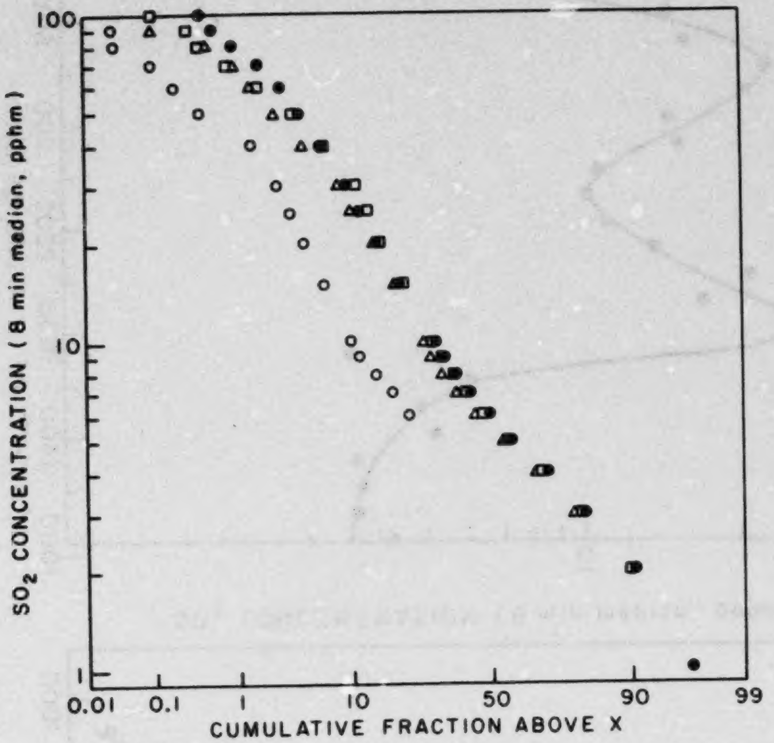

Figure 9.6. Cumulative frequency distributions of 8-min. median $SO_2$ concentrations, normalized to same geometric mean, 1975 season. ○ = plot A; Δ = plot B; □ = plot C; ● = plot D.

The peaks observed during the season for various averaging times are presented in Table 9.6, and compared with relevant State and Federal Standards in Table 9.7. Sixteen-hour averages were used to approximate 24-hour averages. The only plot on which the Federal Secondary Standard was exceeded was plot D, while the Montana Standards were exceeded on all plots receiving $SO_2$ input.

The diurnal variation of $SO_2$ for a typical 24-hour period during the summer is shown in Figure 9.7. $SO_2$ concentrations usually peaked during the night and were low during the day. A different pattern was evident during late summer and early autumn (Figure 9.8), with peaks occurring early in the morning and late in the afternoon. This pattern is similar to that observed for the prototype system (Lee, Lewis and Body, 1975).

The variation of the GM of 8-min. medians with time of day is shown in Figures 9.9a-e for individual months and in Figure 9.9f for the entire season. These graphs again show that concentrations were generally lowest at mid-day, and that the pattern of $SO_2$ variation was similar across the plots.

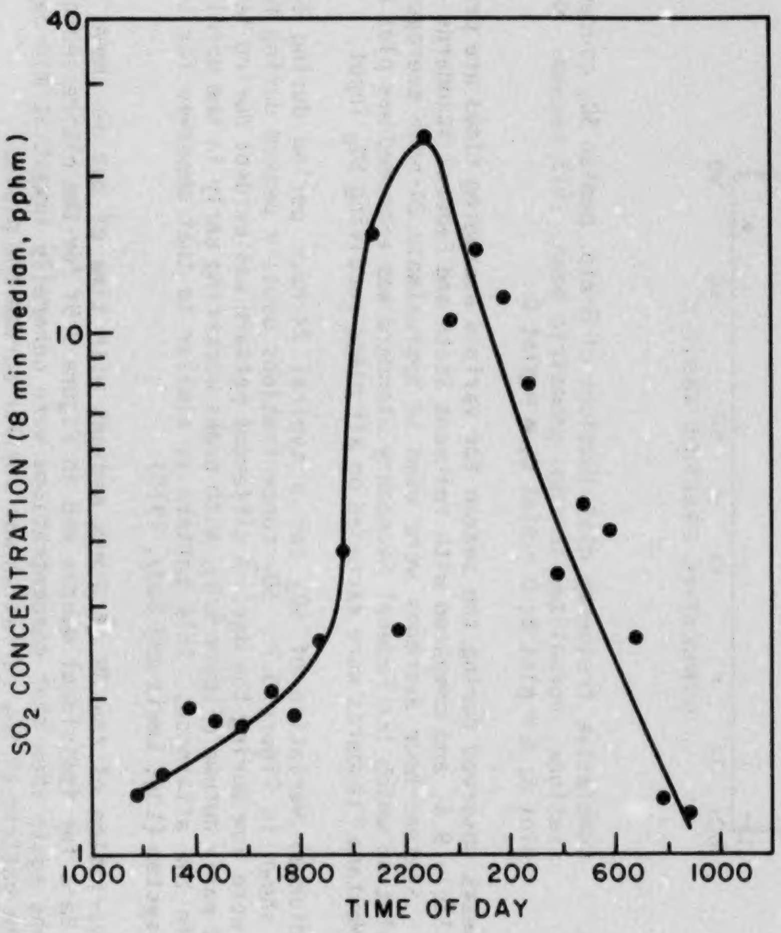

Figure 9.7. Variation of SO$_2$ concentration at location c, plot D, for 24 hours following 10 a.m., August 14, 1975.

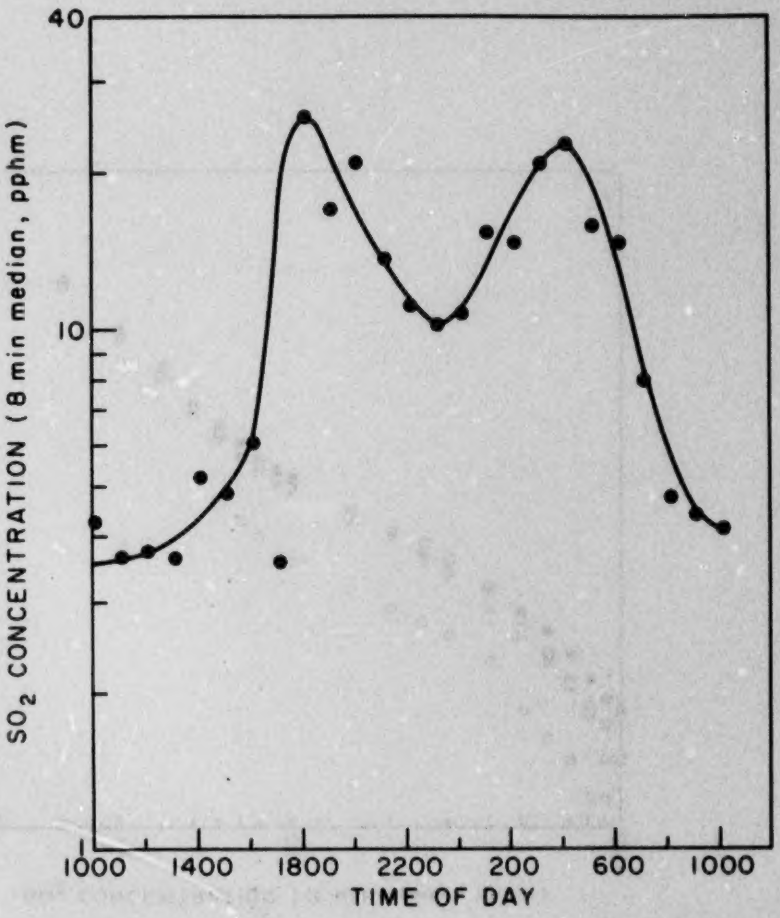

Figure 9.8. Variation of SO$_2$ concentration at location c, plot D, for 24 hours following 10 a.m., September 14, 1975.

Diurnal patterns of pollutant $SO_2$ concentration from stationary point sources and in urban environments are frequently similar to that produced by this system (Holzworth, 1973; Le Quinio, 1973; Raynor et al, 1974; Smith, 1968; Saito and Mizoguchi, 1973). Such variations in air pollutant concentrations are due to (a) variations in source strengths that in turn may result from daily cycles in human activity (Garnett, 1973; Holzworth, 1973); (b) variations in transport wind speeds and directions, atmospheric diffusion and interactions (Cormier, 1974; Fukuoka, 1973; Garnett, 1973; Holzworth, 1973; Lomaya and Tsintsadze, 1974; Martin, 1974; Smith, 1968). All of these vary with weather and season (Balabuyev et al., 1973; Druilhet and Fontan, 1973; Fukuoka, 1973; Sändig and Sändig, 1973). Atmospheric dilution is frequently greatest during the day and least at night. This may result in one or more daytime minimal and a nocturnal maximum in pollutant concentration (Holzworth, 1973).

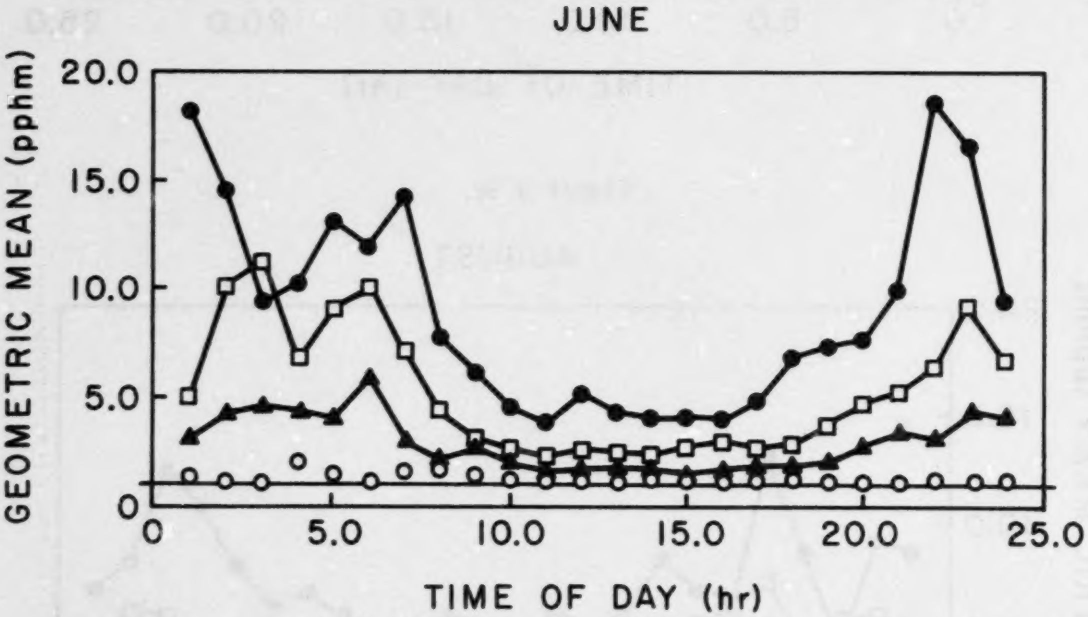

Figure 9.9a. Variation of geometric means of $SO_2$ concentration with time of day for the months June through October and for the entire 1975 season. o = plot A; ▲ = plot B; □ = plot C; ● = plot D. Horizontal line at 0.9 pphm represents the detection threshold; all concentrations at or below this level were assigned a value of 0.9 pphm for the purpose of computing geometric means.

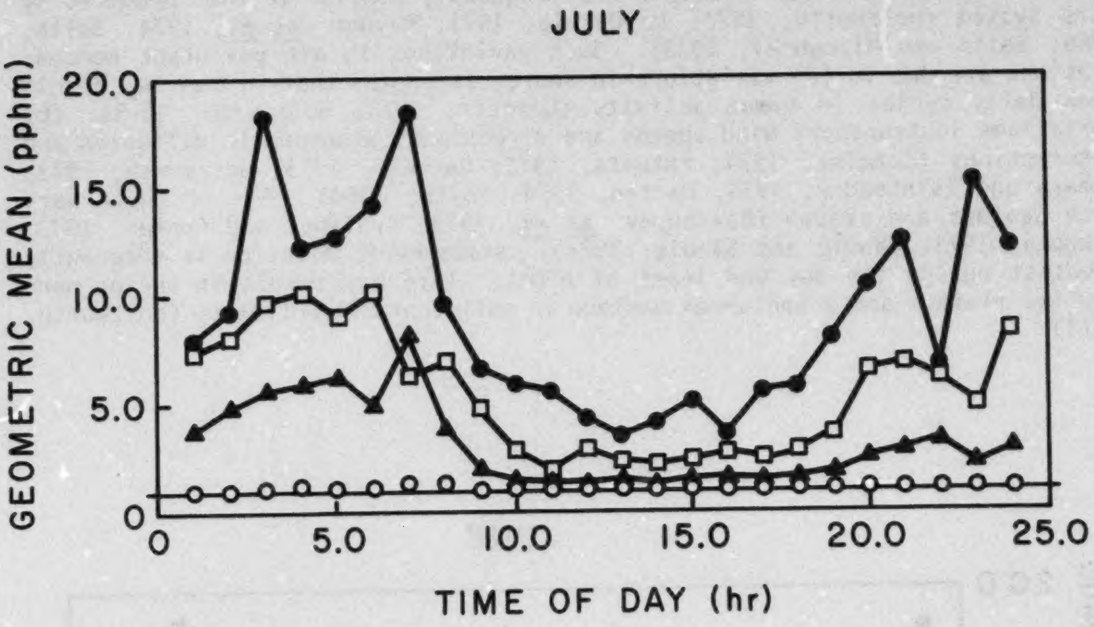

Figure 9.9b.

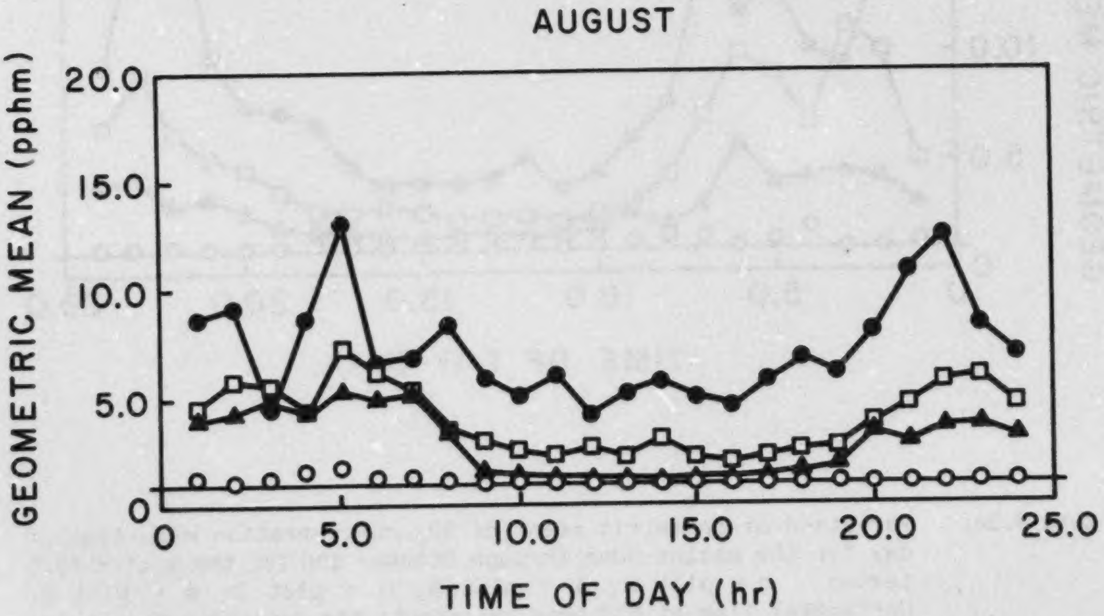

Figure 9.9c.

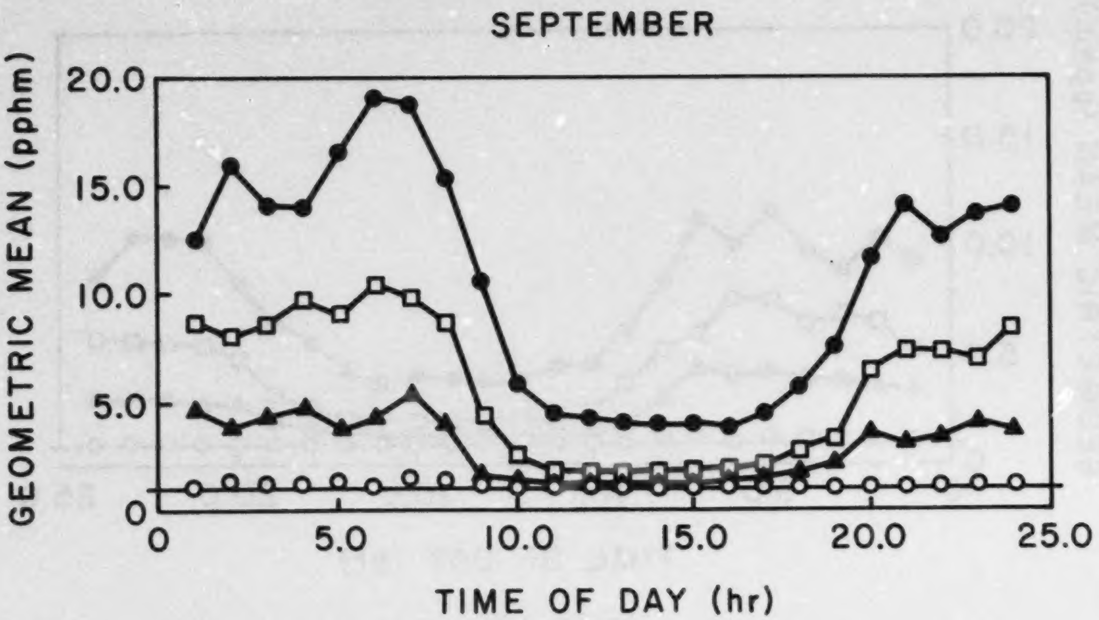

Figure 9.9d.

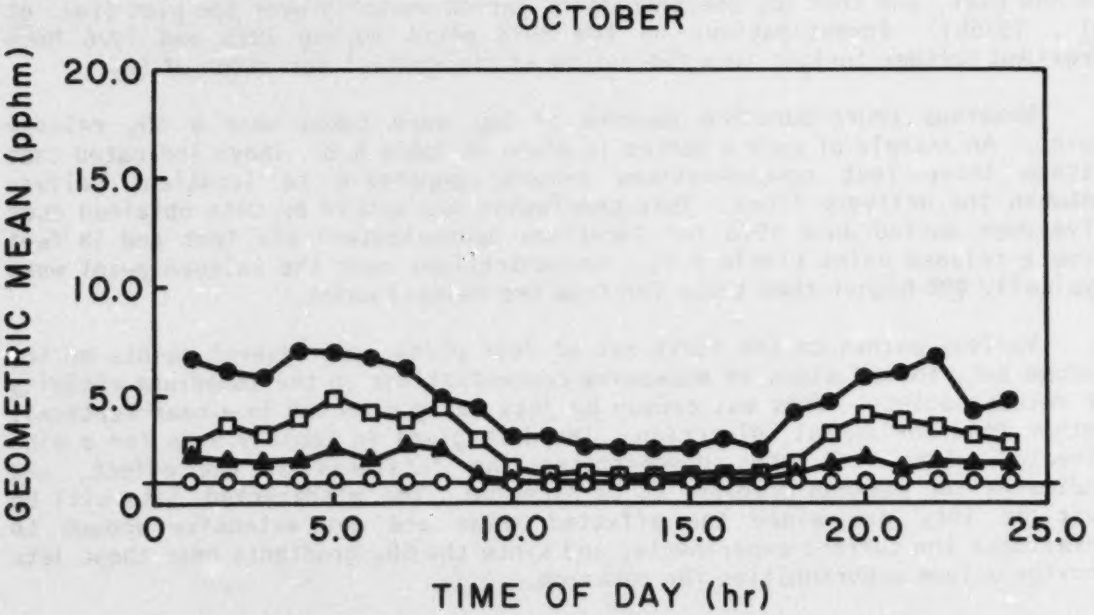

Figure 9.9e.

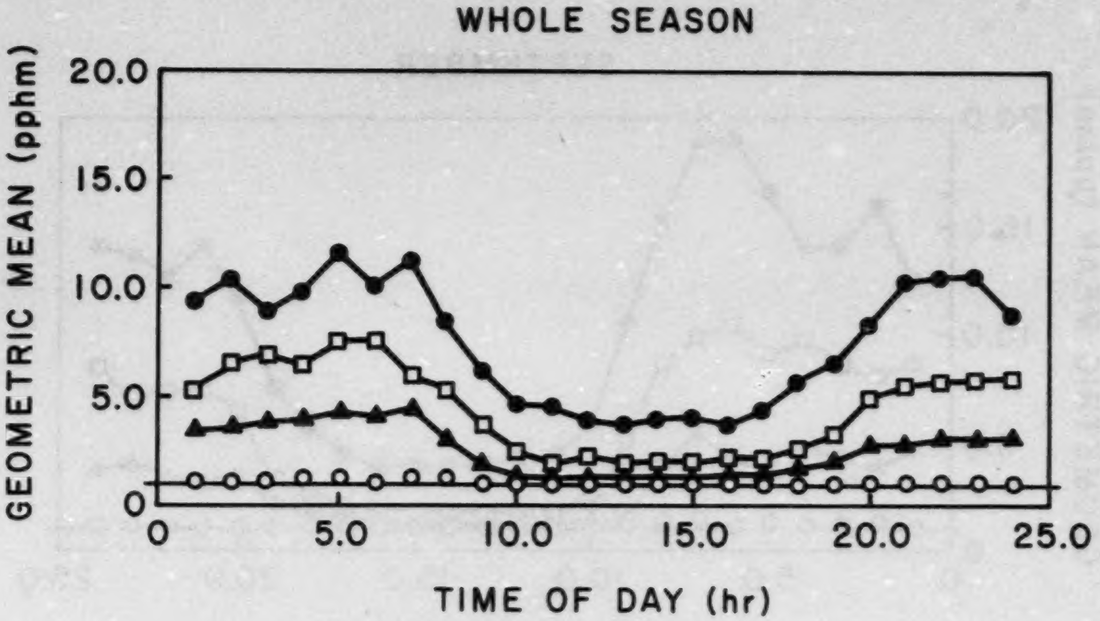

Figure 9.9f.

SPATIAL VARIATION OF $SO_2$

Tests on the prototype system showed that there were no "hot-spots" of $SO_2$ on the plot, and that $SO_2$ concentrations varied smoothly over the plot (Lee et al., 1976b). Investigations on the ZAPS plots during 1975 and 1976 have provided further insight into the nature of the spatial variation of $SO_2$.

Numerous short-duration samples of $SO_2$ were taken near a $SO_2$ release point. An example of such a series is given in Table 9.8. These indicated that within three feet concentrations become comparable to locations halfway between the delivery lines. This conclusion was upheld by data obtained over five days during June 1976 for locations approximately six feet and 18 feet from a release point (Table 9.9). Concentrations near the release point were typically 48% higher than those far from the release point.

Various points on the first set of four plots, and several points on the second set, showed signs of excessive concentrations in the immediate vicinity of release points. This was caused by jets being directed in a near-vertical, rather than horizontal, direction. The data given in Table 9.9 is for a misdirected plume. It thus demonstrates the localness of the effect, and indicates the maximum gradient to be expected. The misdirected jets will be left as they are since the affected areas are not extensive enough to invalidate the current experiments, and since the $SO_2$ gradients near these jets provide unique opportunities for research.

TABLE 9.8. VARIABILITY OF $SO_2$ CONCENTRATION IN THE VICINITY OF A $SO_2$ RELEASE POINT NEAR LOCATION c.

| Horizontal Distance Downwind from Release Point (cm) | Vertical Distance Below Release Point (cm) | $SO_2$ Concentration (10-min. median) (pphm) |
|---|---|---|
| 0 | 30 | 4.3 |
| 0 | 15 | 18.2 |
| 25 | 65 | 3.1 |
| 25 | 30 | 3.1 |
| 25 | 15 | 185.0 |
| 25 | 0 | 5.5 |
| 50 | 0 | 4.2 |
| 100 | 0 | 14.6 |
| 200 | 0 | 5.1 |
| 300 | 0 | 6.1 |

Notes:

1. June 11, 1976.
2. Winds light and variable, 1-2 mph.
3. $SO_2$ concentration at standard location c was approximately 5 pphm.
4. Measurements are for consecutive 10 min. periods separated by 10 min. periods during which the $SO_2$ monitor was zeroed with a $SO_2$ filter.

TABLE 9.9. COMPARISON OF $SO_2$ PARAMETERS 2 METERS AND 6.1 METERS FROM A DELIVERY LINE. June 12-16, 1976.

| Distance From Line (meters) | G.M. (pphm) | Median (pphm) | S.G.D. |
|---|---|---|---|
| 2.0 | 16.7 | 13.0 | 2.6 |
| 6.1 | 11.4 | 8.8 | 2.5 |

A comparison of $SO_2$ concentrations at locations a, b, and c (Figure 9.2) for plot B is given in Table 9.10. These are based on data obtained during most of the 1975 season, and illustrate within-plot variability. The GM's agree to within ± 5%, and the SGD's to within ± 10%. The standard location (c) has an intermediate GM, but exhibits somewhat more variability (highest SGD). This location has the lowest 1-hr peak, an intermediate 3-hr peak, and the highest 16-hr peak. The $SO_2$ concentration at the standard location is typical of $SO_2$ concentrations across the plot.

TABLE 9.10. COMPARISON OF $SO_2$ CONCENTRATIONS ACROSS PLOT B.

| Sample Location | G.M. (8-min medians) (pphm) | S.G.D. (8-min medians) | 1-hr Peak (pphm) | 3-hr Peak (pphm) | 16-hr Peak (pphm) |
|---|---|---|---|---|---|
| a | 2.0 | 2.0 | 43 | 26 | 8 |
| b | 2.2 | 2.2 | 33 | 18 | 8 |
| c | 2.1 | 2.4 | 32 | 20 | 13 |

## SUMMARY AND CONCLUSIONS

The exposure system behaved in a predictable and realistic manner during the 1975 season. Different $SO_2$ distributions were maintained on the four plots, and these had the desired relationships to the Federal Secondary Standard for $SO_2$. Maintenance was routine and not excessively time consuming. The system is a useful and practical tool for determining the effects of $SO_2$ on naturally growing ecosystems.

The behavior of the system is similar in several respects to a system that was developed independently by a French research team (de Cormis, Bonte' and Tisne, 1975). In the French experiments, trees planted in a 2,000 $m^2$ area have been fumigated through 128 release points with $SO_2$ at an average concentration of 3.4 pphm. The control consists of a similar area without a pollution delivery system located 100 m from the fumigation plot. The French system uses a $SO_2$ flow rate of approximately .85 SCFH, or about 80% that of the ZAPS after allowance for differences in plot areas and $SO_2$ concentrations. The concentration varies with meteorological conditions; high $SO_2$ concentrations are, however, prevented by discontinuing fumigation when the concentration reaches a pre-determined value. They conclude that "The discontinuous nature of pollution related to the effect of climatic conditions is not a handicap. On the contrary, this phenomenon is quite similar to those encountered in certain polluted sites which have provided the basis for this study."

## ACKNOWLEDGEMENTS

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## SECTION 10

# FIRST-YEAR EFFECTS OF CONTROLLED SULFUR DIOXIDE FUMIGATION ON A MIXED-GRASS PRAIRIE ECOSYSTEM

by

J. L. Dodd, W. K. Lauenroth, R. K. Heitschmidt and J. W. Leetham

## INTRODUCTION

At the beginning of the Colstrip Coal-fired Power Plant project it was concluded that sulfur dioxide ($SO_2$) was probably the single most important gas of the several effluents expected from the power plant. A field experiment was designed to expose four 0.5 ha grassland study sites to different ambient concentrations of sulfur dioxide on a continuous basis throughout the growing season. The objectives of the experiment were to determine the effects of 2, 5 and 10 pphm monthly median sulfur dioxide concentrations on key structural and functional attributes of the grassland. These concentrations were selected to bracket Federal and State of Montana primary and secondary air quality standards.

The fumigation experiment is located on a grassland site in the Custer National Forest 85 km southeast of Colstrip. At this distance it is presumed to be out of the influence of the Colstrip plume. The site is located at an elevation of about 1,200 m on the divide between the Powder River and the Tongue River drainage basins.

Soils of the study site are Farland silt loam. These are well developed soils and on the site, have an A-horizon about 25 cm thick over a B-horizon that is about 70 cm thick. The climate in this part of the Great Plains is continental with about 75% of the precipitation occurring during the growing season. The annual rainfall at the study site is about 350-400 mm.

The vegetation of the site is typical of the northern mixed grass prairie in that it is dominated by cool season grasses, mainly western wheatgrass and junegrass, and has smaller quantities of other grasses and a variety of forbs and half-shrubs.

Data on various abiotic variables including air and soil temperatures, relative humidity, wind velocity, soil water content, and precipitation were collected for most of the growing season. Growing conditions in the early part of the 1975 season were very favorable with May and June each receiving over 100 mm of precipitation. This is probably well above normal for the area. Soil water was near field capacity until about the first of July and then decreased rapidly and remained low throughout the remainder of the season.

## PHENOLOGY

Previous investigations concerning the effects of $SO_2$ upon the primary producer component of an ecosystem have virtually ignored reproductive processes. The possible effects of air pollutants on pollination, flower set, seed development, and seed dispersal should not be overlooked. Any changes in the time of occurrence of these events as a result of exposure to air pollutants may significantly alter the plant's reproductive capabilities.

The objective of the phenology study was to identify any major differences between treatments in the phenological development of the major plant species.

Fourteen species were observed on a weekly time interval throughout the growing season (Table 10.1). As can be seen, a majority studied were cool season species and, for a considerable portion of these species, phenological development occurred prior to initiation of the continuous fumigations in late May.

TABLE 10.1. SCIENTIFIC AND COMMON NAMES OF THE 14 MAJOR PLANT SPECIES SELECTED FOR PHENOLOGICAL STUDY

| Scientific Name | Common Name |
|---|---|
| Cool season grasses | |
| Agropyron smithii | Western wheatgrass |
| Bromus japonicus | Japanese brome |
| Koeleria cristata | Prairie junegrass |
| Poa secunda | Kentucky bluegrass |
| Stipa comata | Needle-and-thread |
| Warm season grasses | |
| Aristida longiseta | Red-three-awn |
| Bouteloua gracilis | Blue gramma |
| Warm season forbs | |
| Antennaria rosea | Rose pussytoes |
| Psoralea argophylla | Silverleaf scurf pea |
| Cool season forbs | |
| Achillea millifolium | Western yarrow |
| Sphaeralcea coccinea | Scarlet globe mallow |
| Taraxacum officinale | Common dandelion |
| Tragopogon dubius | Yellow salsify |
| Half shrubs | |
| Artemisia frigida | Fringed sagewort |

A 14-stage phenological classification (Table 10.4 in Section I) was utilized to describe the observed phenological status of the selected species within each treatment on each observation date. The recorded status reflected the mean of all plants of the species within a treatment.

Statistical analyses indicated no significant differences between treatments in the time of occurrence of major phenological events. However, a definite trend was noted indicating an advancement in phenological development with increasing $SO_2$ concentration. This trend was evidenced by 10 of the 14 species observed (Appendix).

An example of a species displaying this trend is false salsify, a cool season forb (Figure 10.1). The low and high treatments reached the mature floral bud stage, stage 9, one week prior to the control and medium treatment while all three treatments reached the open flower stage, stage 9, two weeks prior to the control. In addition, the medium and high concentrations began seed dispersal approximately one week prior to the control and low treatments. This trend was reflected in all groups except the half-shrubs in which fringed sagewort was the only species observed.

Although it was concluded from statistical analyses that no major differences occurred in the timing of phenological events as a result of $SO_2$ fumigation, the trend toward advancement with increasing $SO_2$ concentration suggests minor differences may have occurred. Since observations were only conducted approximately every 6 to 7 days, treatment differences of only 2 to 3 days would probably not have been revealed except on every third or fourth observation date. Furthermore, since continuous fumigation was not initiated until after a considerable portion of the phenological progression of the cool season species had occurred, a more dramatic effect might be expected if fumigation were initiated at the beginning of the growing season.

## VISIBLE INJURY

Visible injury to plant tissue resulting from $SO_2$ exposure has been the subject of numerous studies since the early 1930's (Hill and Thomas, 1933; Thomas and Hill, 1935; Nat. Res. Council Con., 1939). The earliest studies primarily focused on describing characteristic patterns of visible injury as exemplified by different species and the effect of this injury on yield in various agronomic crops (Briesley et al., 1950, 1959). More recently, emphasis has been placed on determining the "threshold concentration" inducing visible injury (Thomas, 1956; Daines, 1968). These "threshold concentrations" have been found to vary with changes in concentration, duration of exposure, and abiotic variables such as temperature, relative humidity, and light (Daines, 1968). However, a large majority of these studies have been conducted under controlled laboratory conditions and normally evaluated only acute effect of high concentration short-term exposures. Field-observed chronic visible injury induced by low concentration long-term exposures has not previously been reported for native range species.

The objective of the following research was to identify and describe any visible injury incurred by western wheatgrass as a result of $SO_2$ fumigation.

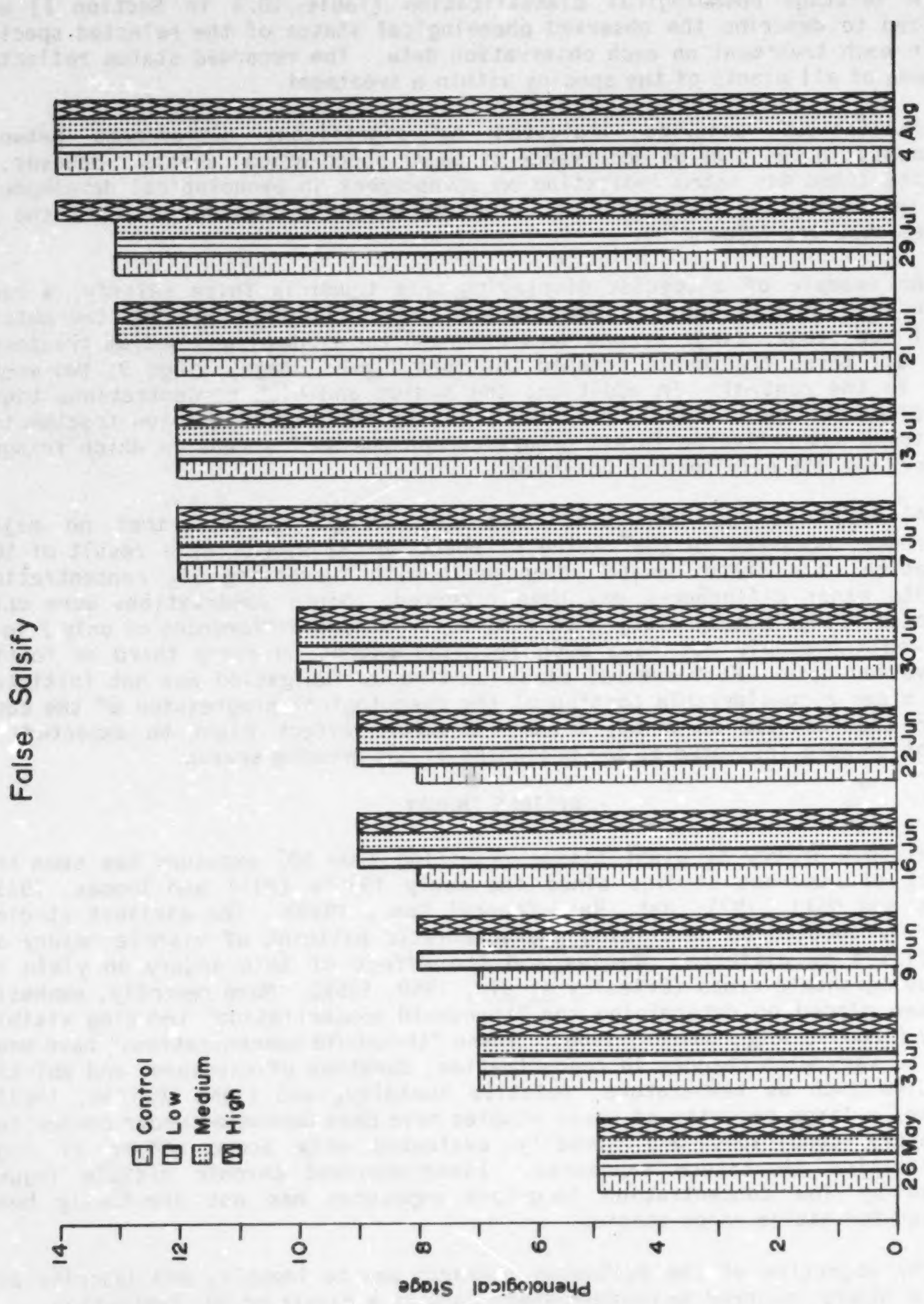

Figure 10.1. Phenological development of false salsify (Tragopogon dubius) for Taylor Creek, 1975.

Western wheatgrass was selected for this facet of the study since it is the dominant species within the fumigation plots and because its morphological characteristics are such that field collection, mounting, and visible injury determinations are simplified.

Four plants were collected outside the perimeter of each harvest quadrat on the June, July, August, and September sample dates. After the quadrat was randomly located, the four closest western wheatgrass plants to the corners of the quadrat were clipped at ground level. Thus, 20 plants were collected per replication and 40 plants per treatment.

After clipping, the leaves were removed and mounted in order of age between two strips of clear adhesive acetate. Each leaf's total surface area and total injured area was then recorded from visual determinations using a guided overlay. Visible injury was defined to include any portion of the leaf exhibiting necrotic condition, including normal leaf senescence. The total surface of missing leaves was considered injured.

An increase in the degree of leaf senescence has previously been reported as the only visible injury detectable in perennial ryegrass plants when exposed throughout a growing season to low concentrations of $SO_2$ (Bell and Clough, 1973). Examination of the leaf surfaces of our sampled plants also revealed no distinguishable pattern of visible injury other than an increase in leaf senescence. Consequently, statistical analyses were run in an attempt to detect differences between the percent of leaf surface necrosis in the control plants and the fumigated plants. Visible injury, resulting from exposure to $SO_2$, as used here, is defined as the necrotic portion of the leaf surface exceeding normal senescence as reflected by comparison of the fumigated plants with the control plants. Leaf injury means for western wheatgrass are presented in Figures 10.2 (by data treatment and leaf age) and 10.3 (by leaf age and treatment date). The Analysis of Variance (ANOVA) utilized to test the differences between treatments across the four dates indicated a significant difference between the LOW concentration and the HIGH concentration with no differences between all other means (Table 10.2). As expected, a significant difference ($P < 0.05$) in total injury between the four dates was also found, but this is a reflection of the normal aging process. Since the last sample date was very late in the growing season, it was felt that normal fall senescence might be masking the effects of the $SO_2$. Therefore, an ANOVA was run encompassing data only from the first three sample dates. This analysis indicated significant treatment differences between the CONTROL and the LOW treatment when compared to the HIGH treatment and between the LOW and the MEDIUM treatments (Table 10.3). It is felt that the LOW treatment exhibited a lower percent of leaf necrosis than the control because of a significantly higher number of leaves per sampled plant on the sample date of 6 August. Thus, with a larger proportion of the total leaf surface composed of younger tissue, a lower mean percent of injury was reflected by each plant. It has been reported that middle-aged leaves are more susceptible to $SO_2$ damage than either older or younger leaves (Guderian and Van Haut, 1968). In an attempt to examine this phenomenon, an ANOVA of only the first four leaves was undertaken.

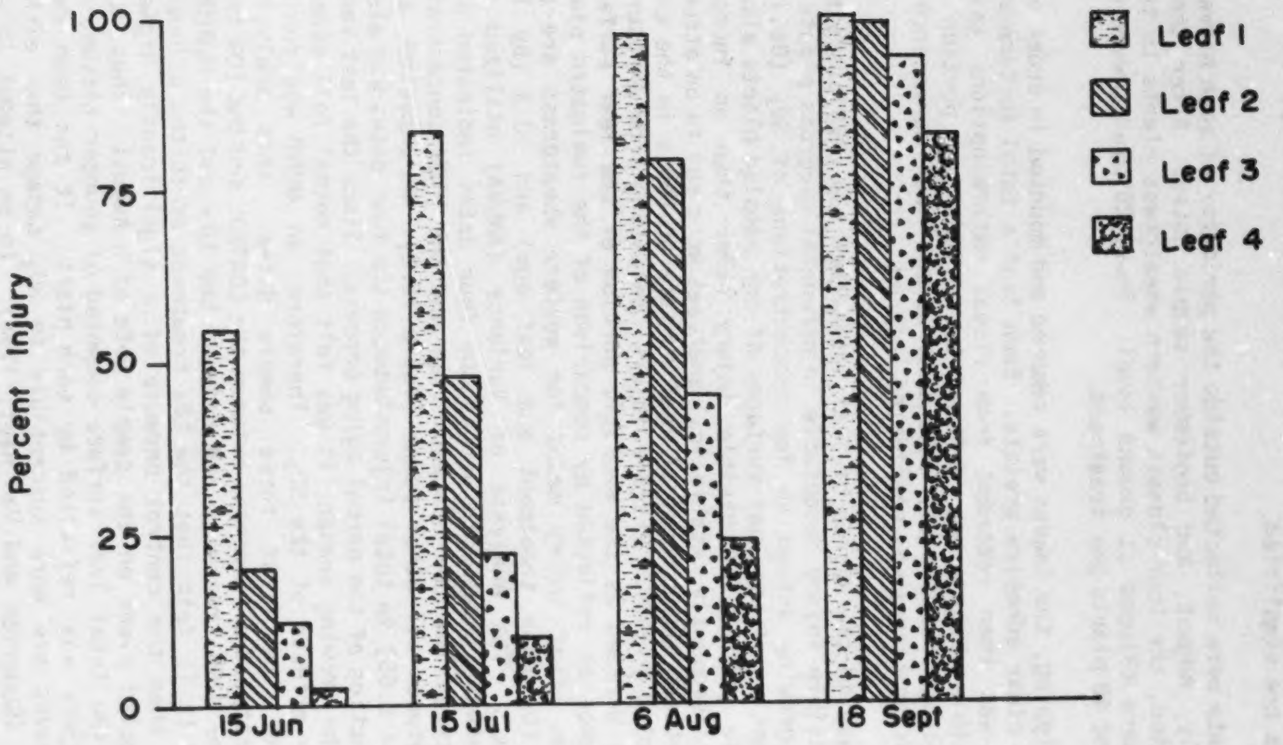

Figure 10.2. Seasonal change in percentage of necrotic leaf surface for first, second, third, and fourth leaves of western wheatgrass, Taylor Creek, 1975.

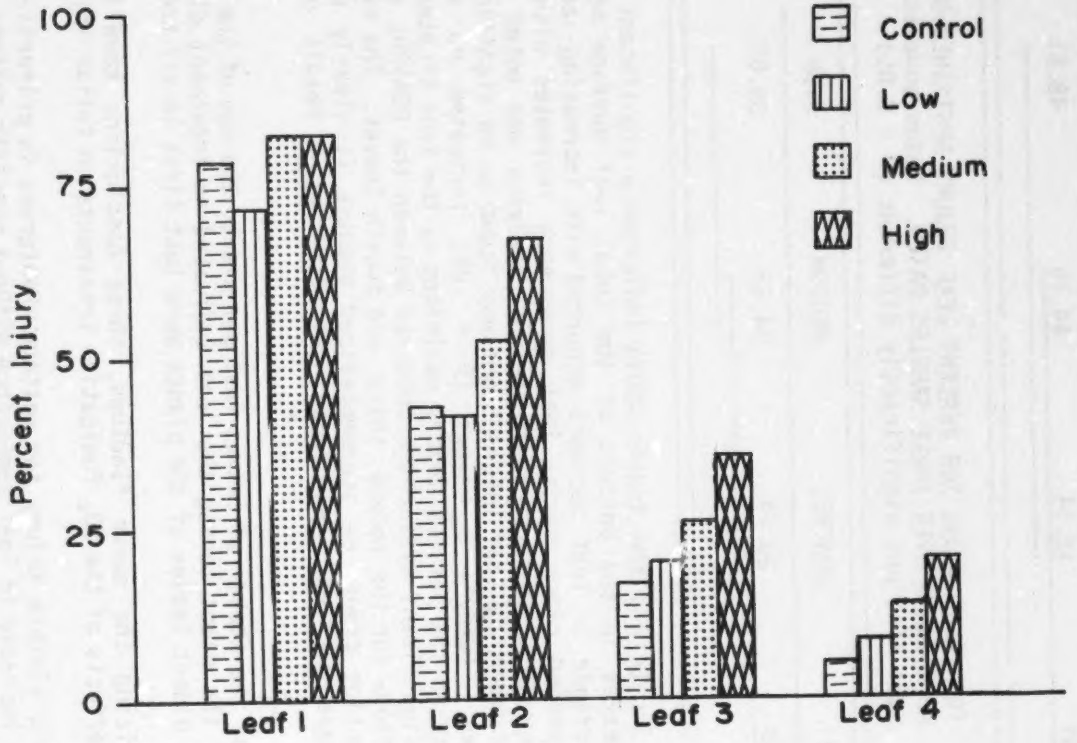

Figure 10.5. Effect of $SO_2$ treatments on leaf injury (percentage of leaf surface necrotic averaged across three dates—June, July, and August) by leaf age, Taylor Creek, 1975.

TABLE 10.2. TREATMENT MEANS FOR PERCENT LEAF INJURY SUSTAINED BY WESTERN WHEATGRASS OVER FOUR SAMPLE DATES. Means underscored by same line are not significantly different ($P < 0.05$).

| LOW | CONTROL | MEDIUM | HIGH |
|---|---|---|---|
| 38.91 | 42.14 | 44.16 | 48.61 |

TABLE 10.3. TREATMENT MEANS FOR PERCENT LEAF INJURY SUSTAINED BY WESTERN WHEATGRASS OVER THREE SAMPLES DATES. Means underscored by same line not significantly different ($P < 0.05$)

| LOW | CONTROL | MEDIUM | HIGH |
|---|---|---|---|
| 26.43 | 29.74 | 34.63 | 38.09 |

Both the 3-date and the 4-date ANOVA indicated a significant difference between all leaves in the percent of the total leaf surface necrotic. A significant increase in leaf necrosis occurred with increasing leaf age, but this is as expected since normal leaf senescence increases with leaf age. However, an important difference in the two analyses was noted in that no significant treatment by leaf interactions was found to be significant in the 3-date analyses. Tukey's Q values ($P = .05$) indicated no significant differences between the percent injury exhibited by the four treatments' first leaves, but a significant difference occurred between the CONTROL and the two HIGH concentrations for the second, third, and fourth leaves. The significance of this interaction cannot be overemphasized because it clearly establishes that an increase in leaf senescence did occur as a result of the $SO_2$ fumigation.

Analyses of the number of leaves lost per plant, the age of the lost leaf, and the age of the plant losing the leaf indicated no treatment differences. In general, the oldest leaves of the plants were lost first in all treatments.

In summarizing the above findings, three conclusions have been drawn concerning the effects of the $SO_2$ fumigation treatments on foliar injury:

1. Chronic visible injury for western wheatgrass is primarily expressed by an increase in leaf senescence without specific pattern.

2. A significant increase in visible injury occurred with increasing $SO_2$ concentrations.

3. A significantly greater amount of injury was incurred by the older leaves than by the younger leaves.

It should be noted that the visible injury conclusions are based on statistically significant differences between the CONTROL and the MEDIUM and HIGH treatments and no differences were found to occur between the CONTROL and the LOW treatment.

The significance of these conclusions, as they relate to a range of ecosystems, remains to be seen. Guderian and Schoenbeck (1971) attempted to distinguish between the two synonyms "damage" and "injury" as they pertain to plant responses resulting from the action of air pollutants. They suggest that "injury" should be considered to include all plant responses to air pollutants while "damage" should only refer to those responses which significantly alter the desired use of the plant. After one season of data collections and analyses, the investigators feel the injury has been detected as reflected by the increase in leaf senescence with increasing $SO_2$ concentration. However, it remains to be seen whether or not damage has occurred.

## PLANT BIOMASS DYNAMICS AND NET PRIMARY PRODUCTIVITY

The objective of this part of the study was to characterize the effects of three levels of sulfur dioxide fumigation on above- and belowground plant biomass dynamics and net primary productivity.

Aboveground biomass was sampled by the harvest method and separated in the field by species and into three categories: live, recent dead, and old dead. Live and recent dead represent growth during the current growing season and old dead is biomass remaining from previous growing seasons. On each of six sample dates, ten randomly located, circular 0.5 $m^2$ quadrats were harvested from each treatment.

Total biomass accumulation by all species was similar with no significant differences among treatments at any time during the season (Figure 10.4). The average growth rate from 15 April to 15 July was 1.4 $g/m^2/day$.

Fumigation was initiated on 1 June and had no measurable effect on biomass accumulation. The point at which we measured a change in the rate of growth was 6 August. This decrease in growth rate appears to be related to a rapid decline in soil water between 15 July and 6 August rather than a response to the fumigation treatments.

The two most important functional groups contributing to total biomass are cool season grasses and cool season forbs. Biomass accumulation by both of these groups was more variable among treatments than total growth, but no significant differences or explainable trends were evident (Figure 10.5). Average growth rate of cool season grasses from 15 April to 15 July was 1.1 $g/m^2/day$. At the time that fumigation was begun, approximately one-third of the cool season grass growth had occurred. Cool season forb growth was greatest for the HIGH concentration treatment and lowest for the LOW treatment with the control falling approximately midway between the two.

Stepping down to a finer level of resolution one can see the responses of the two most important species in the cool season grass group. The first of these is western wheatgrass (Figure 10.6). Western wheatgrass is an important

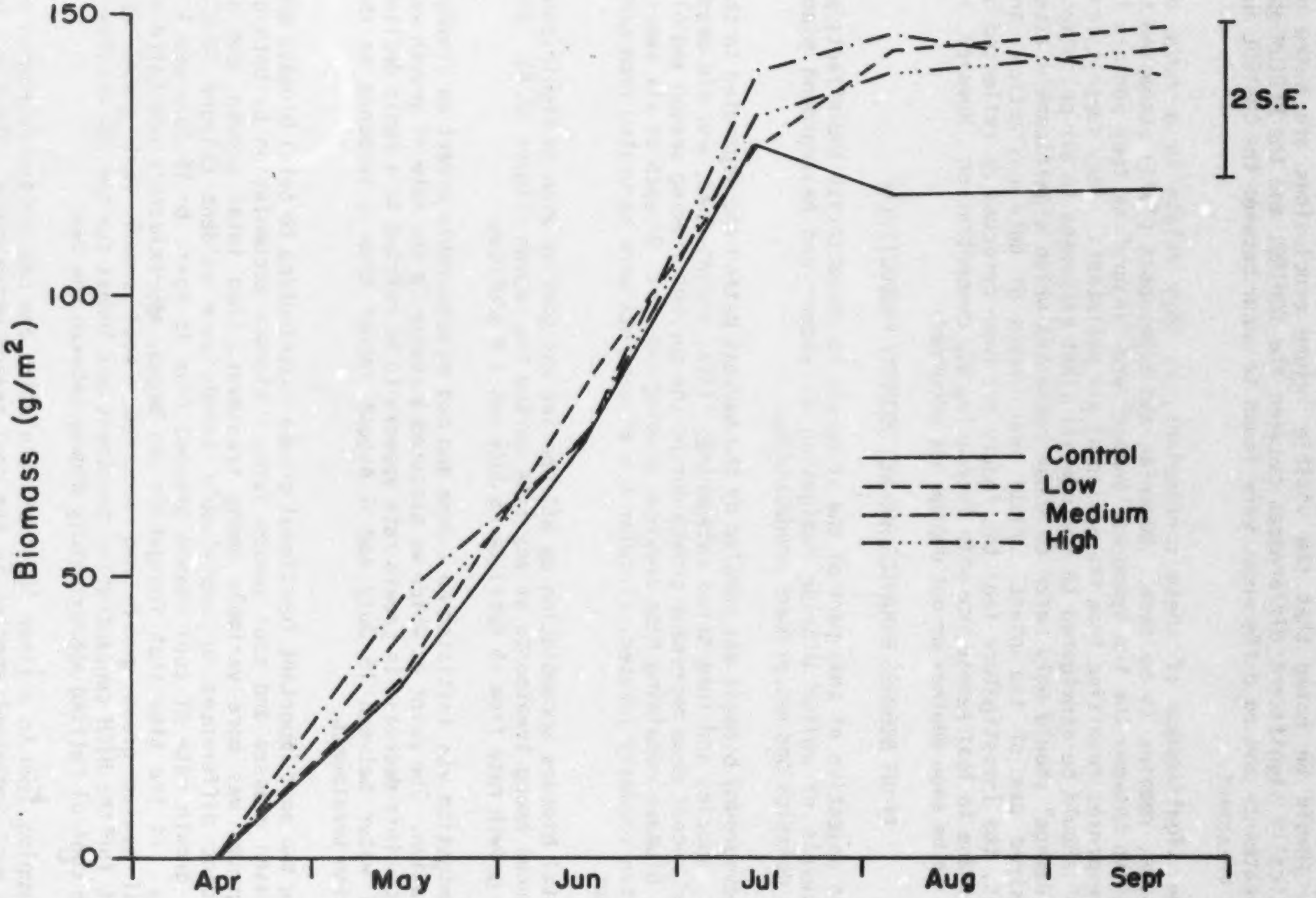

Figure 10.4. Seasonal change in current year's production for all species combined, Taylor Creek, 1975.

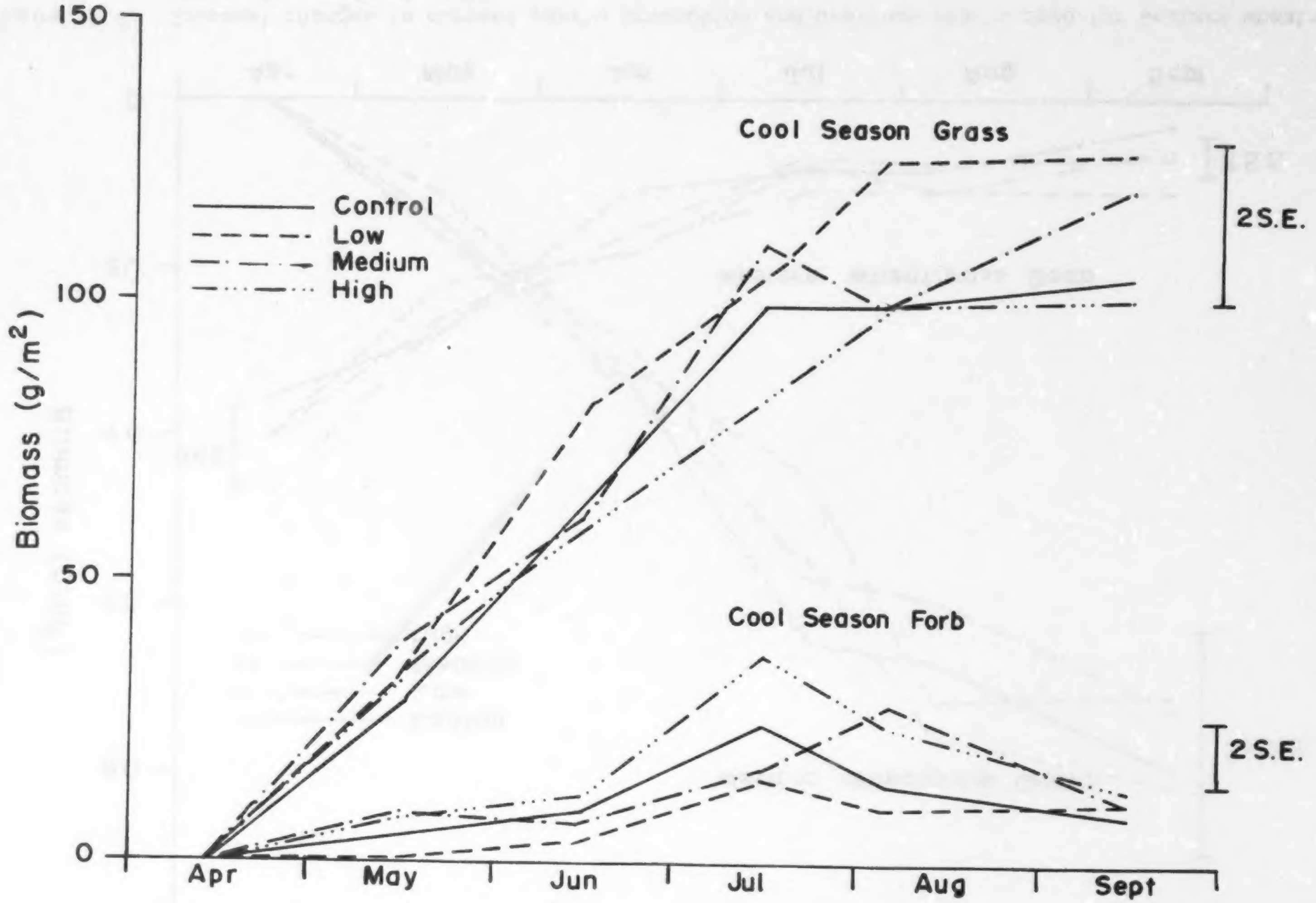

Figure 10.5. Seasonal changes in current year's production for cool season grasses and forbs, Taylor Creek, 1975.

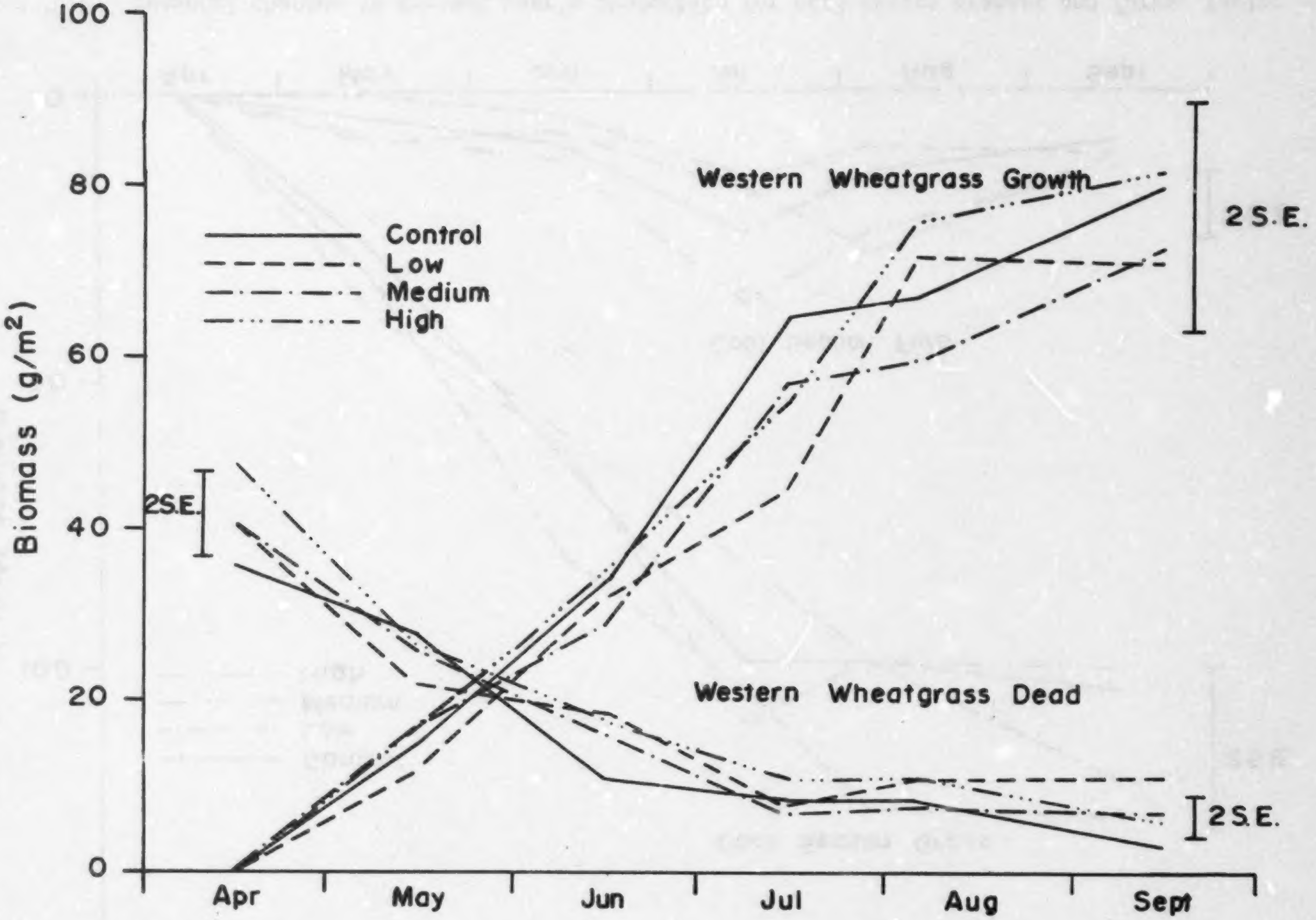

Figure 10.6. Seasonal changes in current year's production and previous year's dead for western wheatgrass, Taylor Creek, 1975.

dominant over much of the northern Great Plains and is probably the most important species to the livestock economy of the region. The growth rate of western wheatgrass averaged 0.6 g/m$^2$/day from 15 April to 15 July and 0.5 g/m$^2$/day for the entire period of measurement.

While these data do not demonstrate treatment effects, they do represent a solid characterization of the dynamics of growth and disappearance to dead material during the 1975 growing season. Additionally the May sample of live and recent dead biomass and the April and May samples of old dead indicate high degree homogeneity of western wheatgrass biomass across treatments. The importance of this homogeneity relates first to the question of whether significant effects of fumigation on the growth of western wheatgrass occurred during 1975 that were offset by initial differences in populations among treatments. These data indicate that this was not the case. Secondly, the homogeneity of western wheatgrass biomass during the first year of treatment will strengthen our conclusions about differences should they occur during the second or third years of treatment.

Prairie junegrass is the second important cool season grass on the experimental plots (Figure 10.7). Several of the differences in prairie junegrass biomass accumulation among treatments are significant although these differences have not been explained in terms of treatment effects. The data for old dead biomass on the first sample date indicate inherent variability in prairie junegrass populations among treatments and at this time differences in prairie junegrass biomass accumulation are attributed to this variability.

Belowground biomass was sampled to a depth of 20 cm by the harvest method. Included in these samples were crowns, rhizomes, and roots. Crowns and rhizomes were separated by hand and represent primarily live tissue while root biomass includes all belowground organic material greater than 0.5 mm$^2$.

Total root and rhizome biomass were not significantly different among treatments on any of the sample dates (Figure 10.8). The most perplexing aspect of these data are the two different trends. All of the fumigated treatments represent one trend while the control exhibits a different trend. There is evidence from the literature that exposure to ozone may reduce root reserves. The action of ozone and $SO_2$ may be similar (Tingey, 1974) and therefore explain these trends, but at this point conclusions based on one year's data cannot be made. Crown biomass was similar among treatments and essentially constant at approximately 60 g/m$^2$.

Total roots and rhizomes were separated into two depth increments of roots and rhizomes alone (Figure 10.9). It is evident that the major differences in belowground biomass occurred in the 0-10 cm layer and did not occur until after fumigation was initiated. Trends in root biomass were similar among treatments between the April and May sample dates and different for all other dates. Again, these differences cannot be satisfactorily explained with a single year's data.

Root biomass in the 10-20 cm layer was essentially constant throughout the growing season at approximately 100 g/m$^2$. Rhizomes also exhibited only minor fluctuations and averaged 25 g/m$^2$.

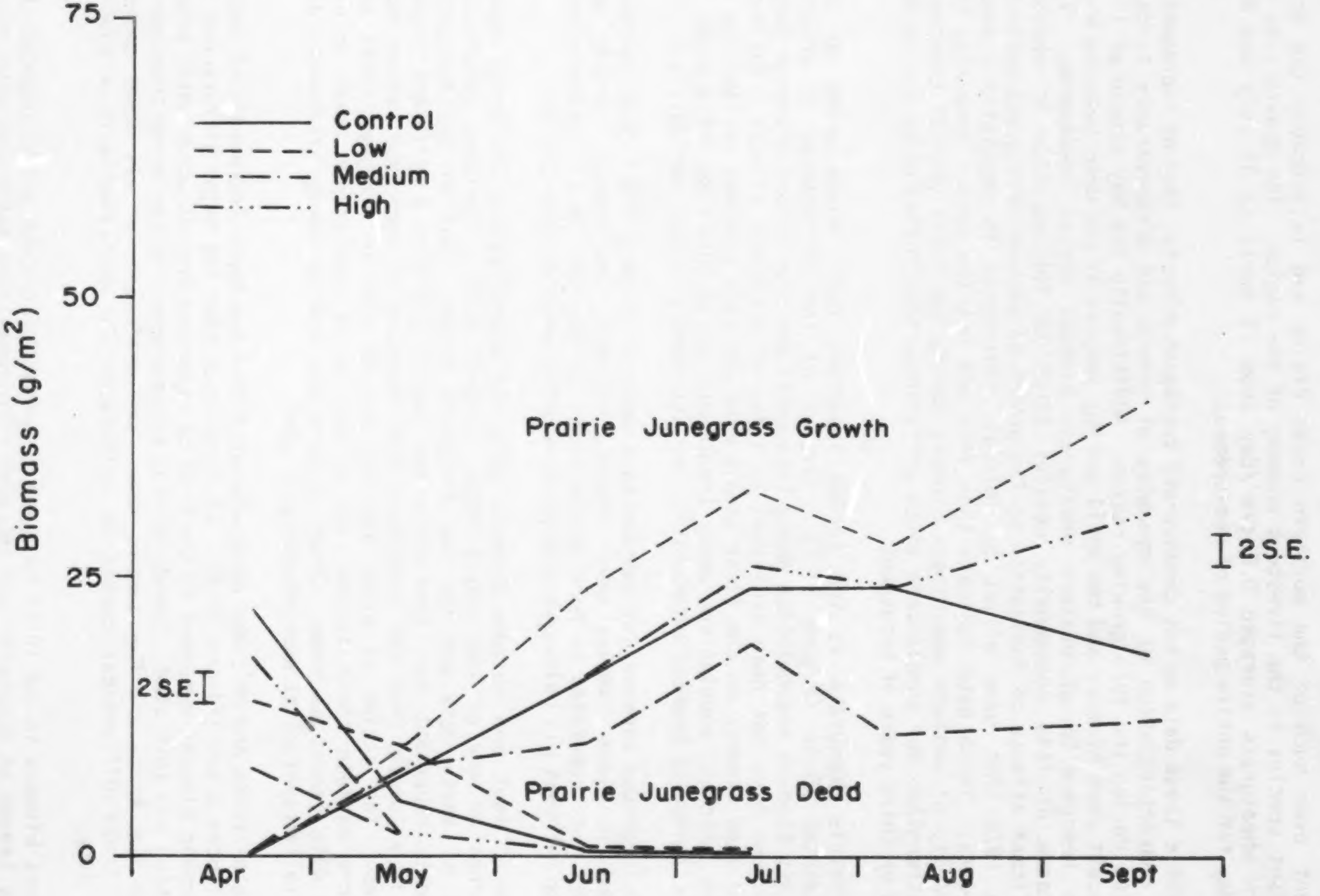

Figure 10.7. Seasonal change in current year's production and previous year's dead for prairie junegrass, Taylor Creek, 1975.

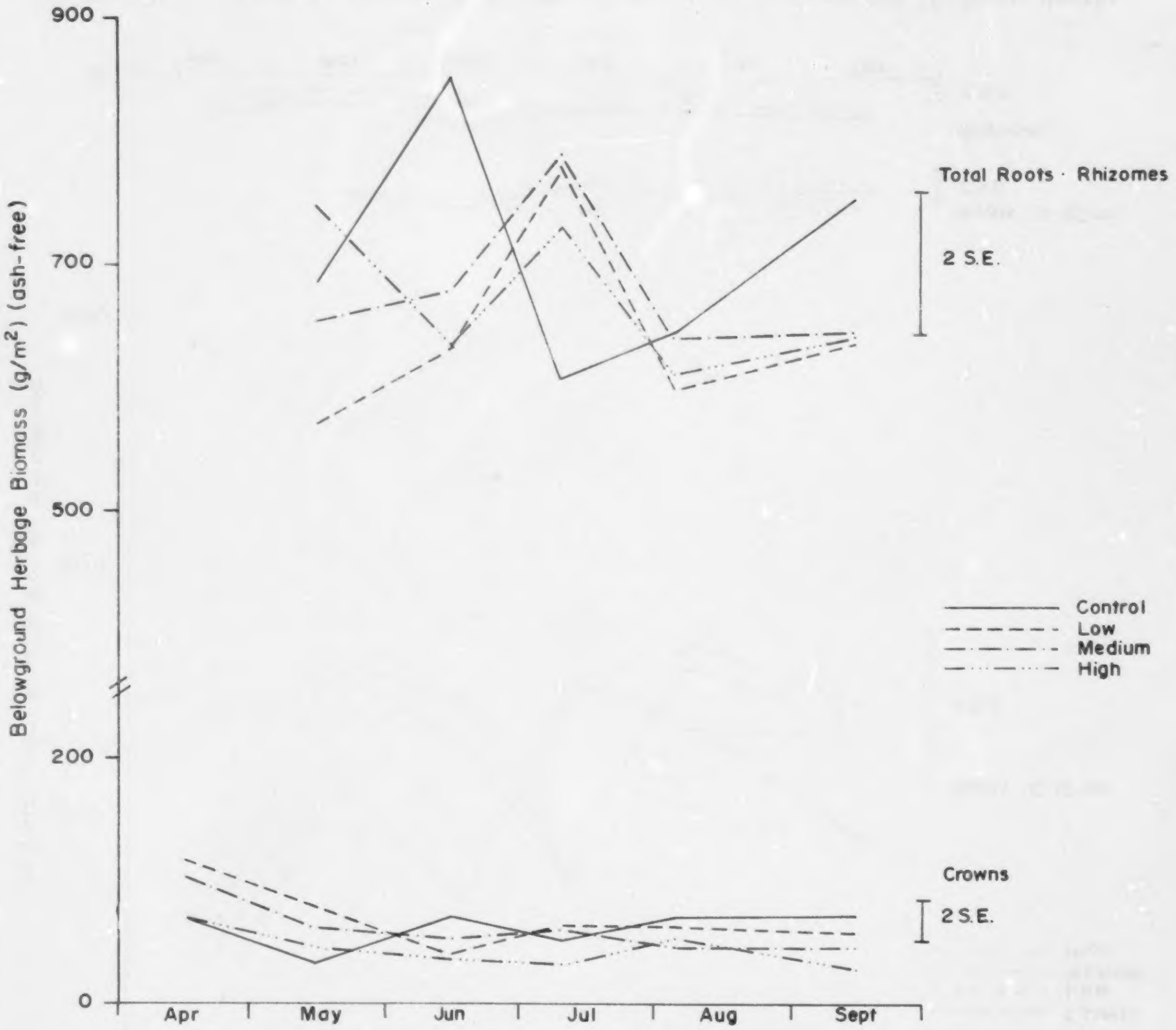

Figure 10.8. Seasonal changes in biomass of crowns and roots plus rhizomes (0.020 cm), Taylor Creek, 1975.

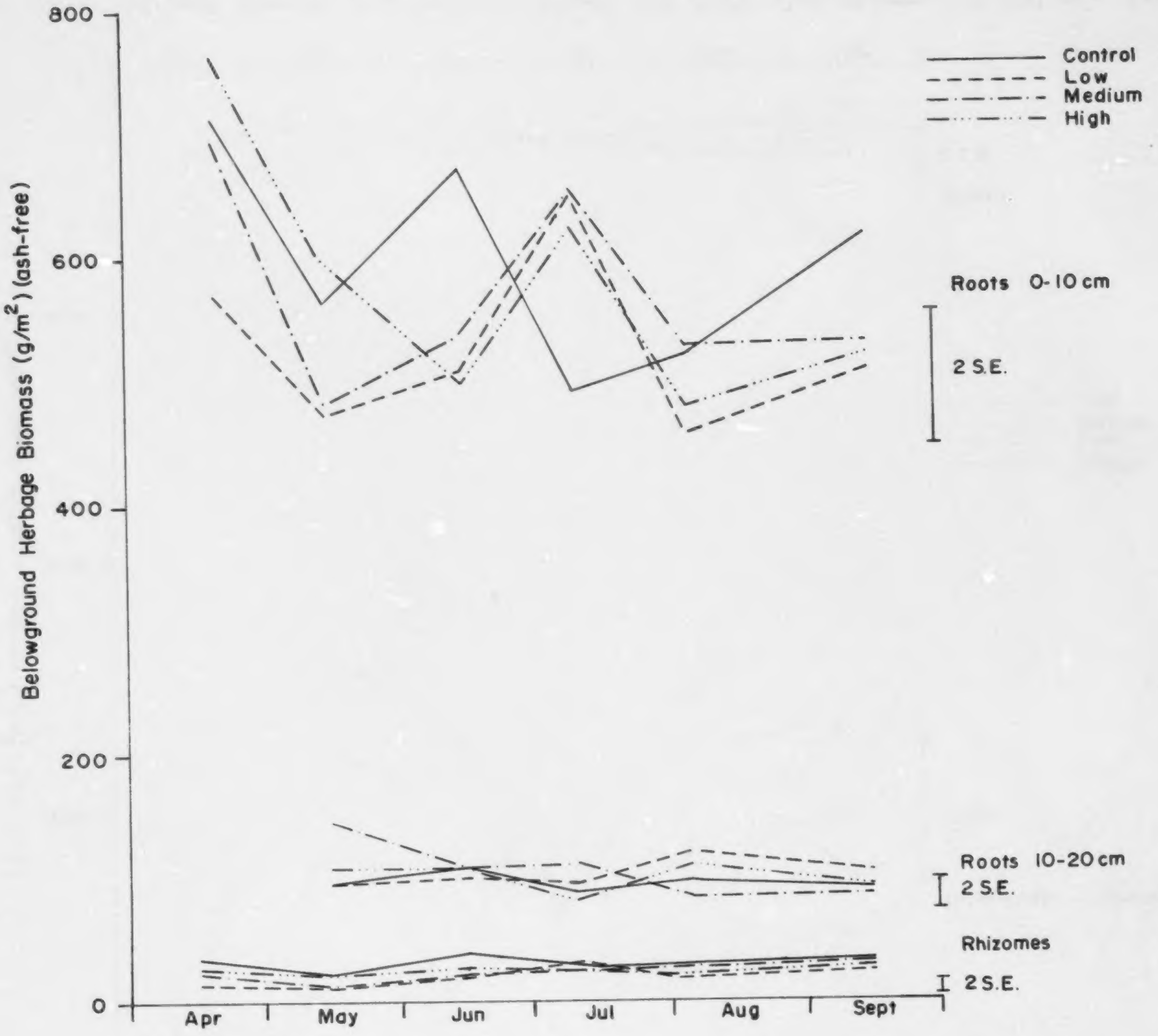

Figure 10.9. Seasonal change in biomass of roots at 0-10 cm and 10-20-cm depths.

Since the results of the belowground biomass sampling are still in question estimates of belowground net accumulation have not been made. Aboveground net primary productivity was calculated by summing peak live plus recent dead biomass by functional groups (Figure 10.10). At first glance it appears that primary productivity increased with increasing $SO_2$ concentration. More careful observation reveals that the higher values for the MEDIUM and HIGH treatments were the result of composition differences. Both of these treatments had measurable contributions of half shrubs while they were only sampled in trace amounts for the CONTROL and LOW treatments. Comparison of aboveground productivity excluding half shrubs shows no major differences among treatments.

In summarizing these results it is emphasized that no significant differences in seasonal biomass dynamics or net primary productivity were found as a result of one year of sulfur dioxide fumigation. There are several reasons why this was not an unexpected result. First, fumigation was not begun until approximately one-third of the total plant growth had occurred. Second, the major primary producers on the experimental plots are grasses and as a group they are generally considered to be less sensitive to $SO_2$ than other plants (Hill et al., 1974). Finally since the sample methods only yield interpretable data on the more abundant species, many significant changes could have occurred in populations of species comprising the forb group that the investigators could not detect. In reference to this last point, sample techniques have been modified for the 1976 growing season and a greater degree of precision for these data is anticipated.

## LITTER DYNAMICS

As shown in Table 10.4 the influence of season and treatment on litter standing crop estimates are unclear. It appears that litter increased between April and May and again between July and August. Differences in litter standing crop resulting from the $SO_2$ stress are not consistent and are probably not statistically significant.

## SOIL RESPIRATION

Soil respiration was estimated utilizing four samples per replication with two replications per treatment on four sample dates (Table 10.5). Difficulties encountered in the laboratory processing of samples prevented early season sampling. The higher rates encountered on 15 August at Taylor Creek and 18 August at Colstrip are attributed to rainfall events immediately preceding the sampling period.

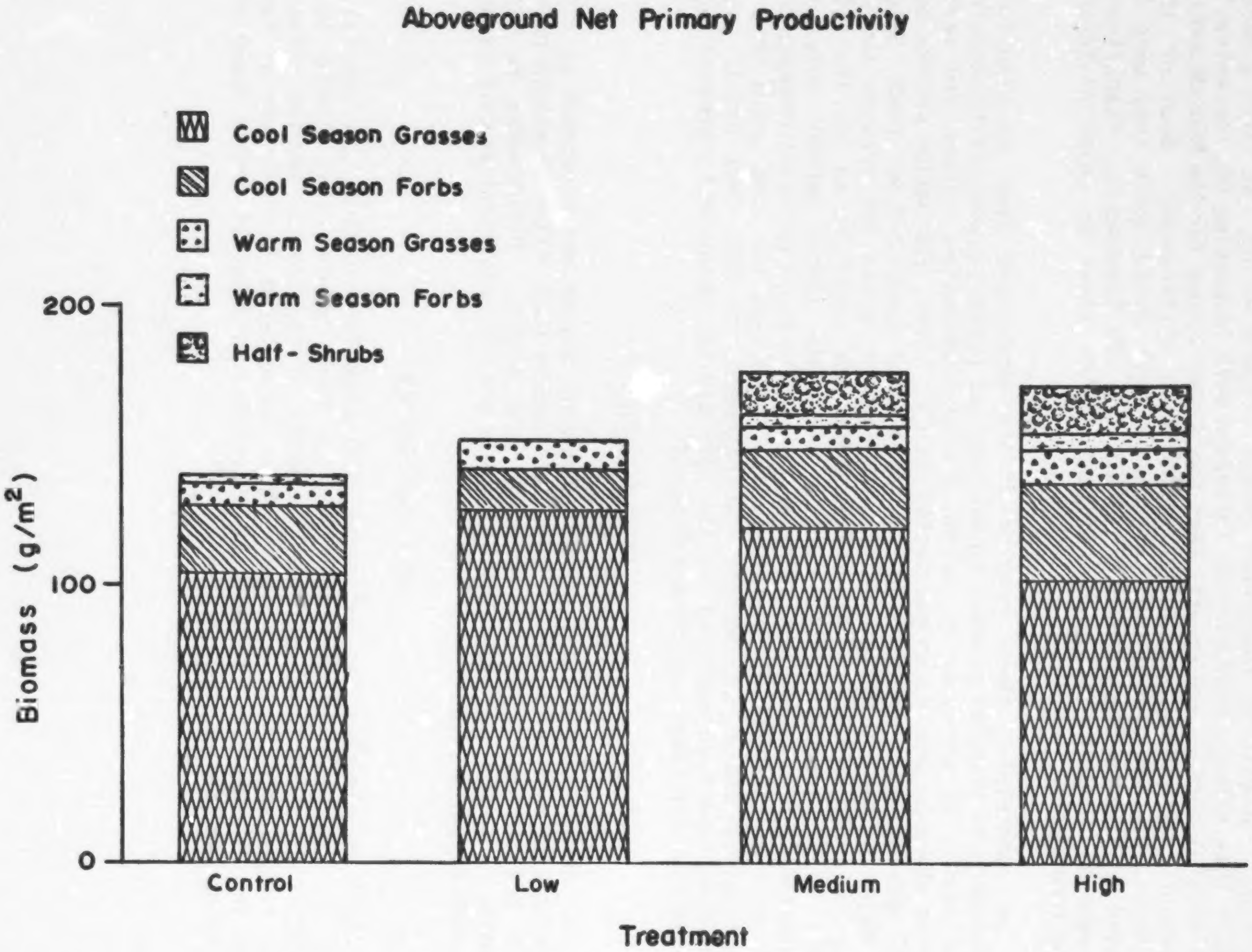

Figure 10.10. Aboveground net primary productivity for Taylor Creek study sites, 1975. (Contributions by functional groups represent peaks of current year's production.)

TABLE 10.4. INTRASEASONAL DYNAMICS OF LITTER STANDING CROP ($\bar{x}$ ± SE, Oven-dry Ash-free g/$m^2$) FOR TAYLOR CREEK SITES (A = CONTROL, B = LOW, C = MEDIUM, D = HIGH).

| Treatment | April 20 | May 5 | June 15 | July 13 | August 13 | September 18 | Season Average |
|---|---|---|---|---|---|---|---|
| A | 112 ± 6 | 102 ± 16 | 154 ± 8 | 123 ± 5 | 172 ± 17 | 145 ± 20 | 138 |
| B | 112 ± 21 | 174 ± 21 | 137 ± 14 | 155 ± 17 | 192 ± 15 | 149 ± 15 | 153 |
| C | 135 ± 16 | 168 ± 24 | 178 ± 17 | 155 ± 18 | 193 ± 17 | 193 ± 17 | 170 |
| D | 39 ± 7 | 179 ± 23 | 124 ± 12 | 154 ± 16 | 189 ± 22 | 100 ± 33 | 148 |
| Average | 100 | 156 | 151 | 147 | 187 | 175 | |

TABLE 10.5. SOIL RESPIRATION TREATMENT MEANS (mg $CO_2/m^2$/day) AND STANDARD ERROR OF ESTIMATE FOR THE FOUR TAYLOR CREEK TREATMENTS ON FOUR DATES, 1975 (A = CONTROL, B = LOW, C = MEDIUM, D = HIGH).

| Date | A | B | C | D |
|---|---|---|---|---|
| 2 August | 5.12 ± .28 | 5.43 ± .21 | 5.72 ± .23 | 5.79 ± .50 |
| 8 August | 4.38 ± .19 | 4.46 ± .16 | 4.00 ± .38 | 4.10 ± .31 |
| 15 August[1/] | 6.92 ± .20 | 6.43 ± .12 | 6.69 ± .29 | 6.81 ± .26 |
| 27 August | 5.97 ± .56 | 4.72 ± .20 | 4.01 ± .34 | 4.18 ± .52 |

[1/] Mean of one replication.

Although final analysis is not complete the 27 August sample at Taylor Creek indicates a significant difference exists between the control (A) and the $SO_2$ treatments. If this difference is found to be real, two obvious explanations exist: (1) reduction in microbial activity because of $SO_2$ treatment; and (2) a reduction in root respiration because of $SO_2$ treatment. More intensive sampling is scheduled for 1976 in anticipation of ascertaining the seasonal dynamics of all sites and treatment differences at Taylor Creek.

## LITTER BAG DECOMPOSITION

The rate of decomposition of native litter was investigated utilizing approximately 5 g of dead western wheatgrass, Agropyron smithii, placed in a mesh bag on the soil surface and retrieved at various time throughout the growing season. The means of the percentage of organic matter decomposed from 10 samples per replicate (two replicates per treatment) are presented in Figure 10.11.

Although statistical analysis has not been completed, two trends are noted from the data. As expected, the maximum rate of decomposition within all treatments occurred in the early part of the growing season. This is attributed to the higher moisture conditions and, thus, favorable for decomposition. The second trend noted is the higher amount of decomposition within the control when compared to the $SO_2$ treatments. This trend suggests a reduction in microbial activity may have occurred because of the $SO_2$ fumigation. However, since the differences between treatments are essentially the same on all dates it is questionable whether the differences can be attributed to $SO_2$ fumigation. Since the fumigations were not initiated until 1 June, the differences found on 10 June may be attributable to site differences. If one hypothesizes that the decomposers are very sensitive to $SO_2$ these differences may have developed from the $SO_2$ fumigation. This hypothesis is supported by the minimal difference noted between the $SO_2$ treatments by the 9 August sample date. The significance of replication of the above investigation in 1976 cannot be overemphasized in light of the 1975 results.

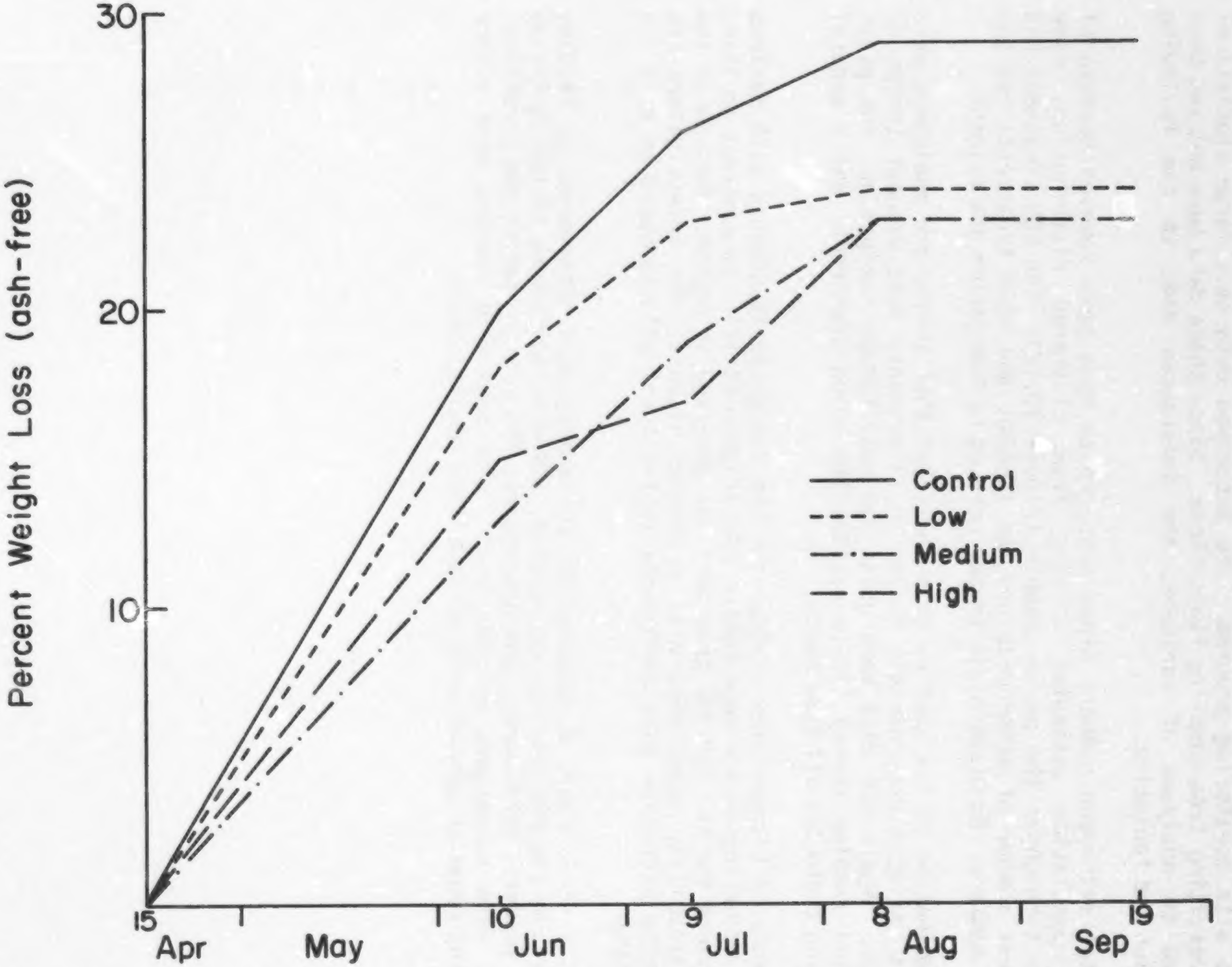

Figure 10.11. Decomposition of Agropyron smithii dead shoot material in nylon mesh litter bags for Taylor Creek study sites, 1975. (Litter bags were placed in field 15 April and subsets were retrieved on 10 June, 9 July, 8 August, and 19 September.)

## ARTHROPOD POPULATION AND BIOMASS DYNAMICS

Above- and belowground arthropods were collected on six dates in 1975. Harvest and extraction techniques were the same as those described for the Colstrip site monitoring program. The arthropod data have large statistical errors resulting from sampling limitations. Since these data have not yet been subjected to analyses of variance, the inferences made in the following discussion are tentative.

Total arthropod numbers minus ants, which have been removed because of erratic population estimates arising from clustered distribution, were variable throughout the period sample (Figure 10.12). The LOW treatment had the fewest number of arthropods and the CONTROL and HIGH treatments had the largest numbers. No discernible trends related to fumigation are evident.

Examination of the species data indicated that thrips and mealybugs were the most variable components. Figure 10.13 presents data without thrips or mealybugs. Again the data were quite variable before fumigation, but post-fumigation samples showed little variability among treatments and a general decreasing trend for all treatments.

Trends in biomass were similar for the fumigated treatments with maximum biomass occurring on the June sample data (Figure 10.14). In contrast to this peak biomass for the CONTROL treatment was observed in August. Because of the large variability associated with arthropod biomass, the investigators are hesitant to attribute this difference to treatment effects based on only one year's data.

Table 10.6 shows a summary of all aboveground arthropods by feeding strategy. Of the 251 species encountered, 50% were herbivores including tissue and sap feeders, nectivores, and granviores; 25% were predators and parasites; and 10% were scavengers or omnivores. The remaining species were either nonfeeding pupae or species with unidentified feeding habits.

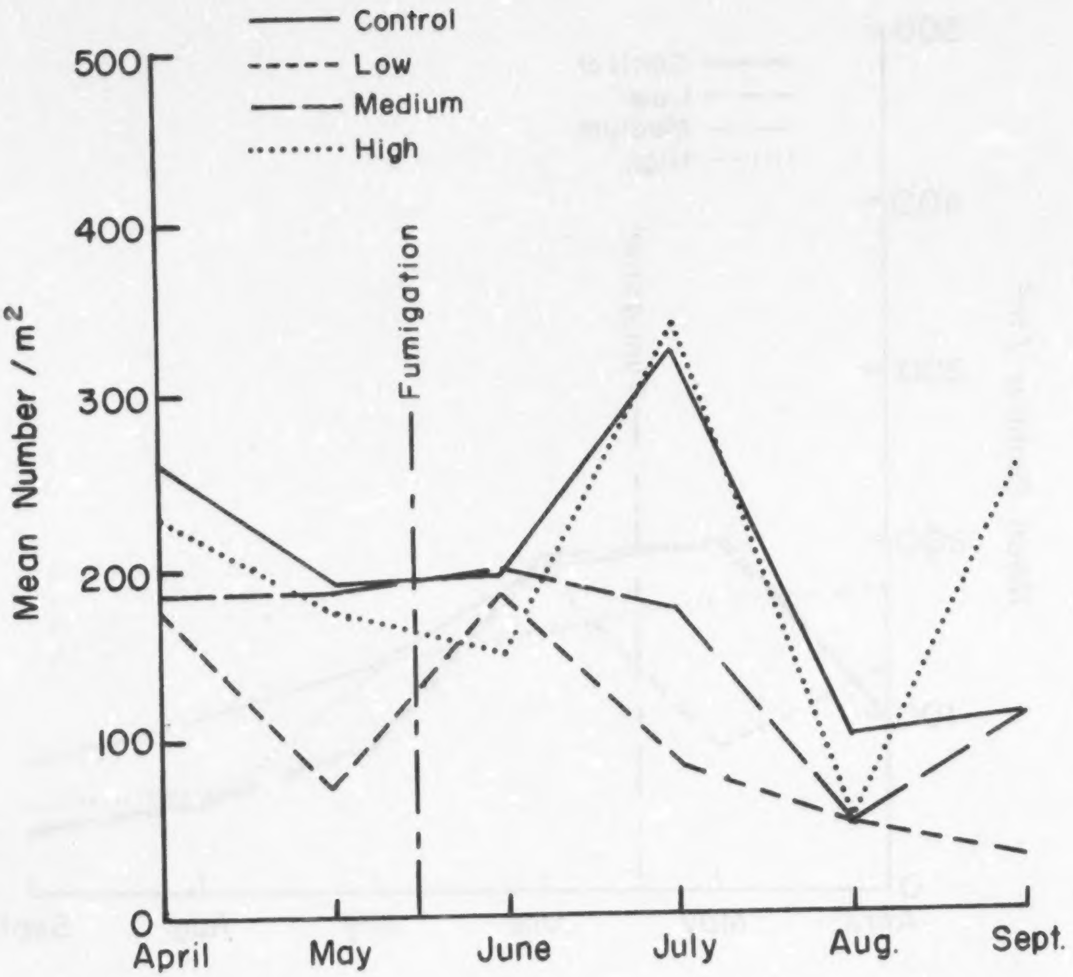

Figure 10.12. Seasonal abundance of total aboveground arthropods for Taylor Creek, 1975.

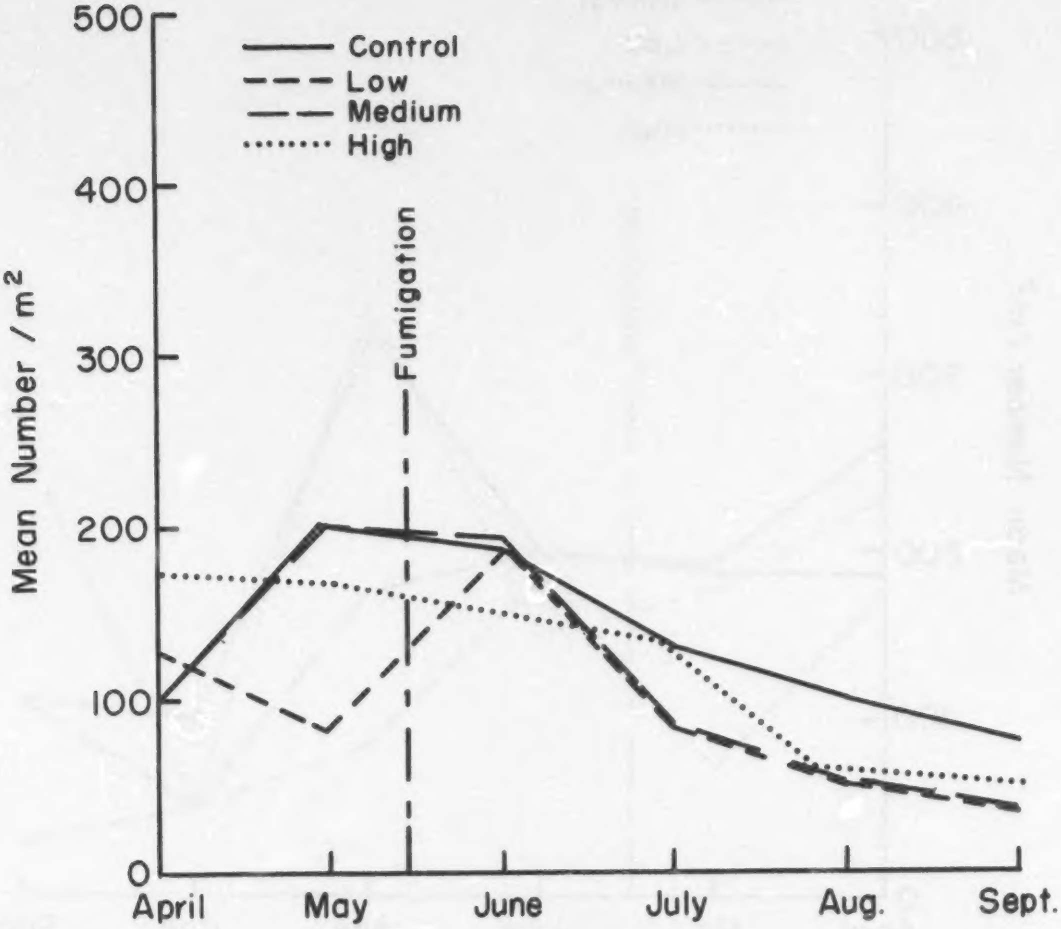

Figure 10.13. Seasonal abundance of total aboveground arthropods excluding ants, Taylor Creek, 1975.

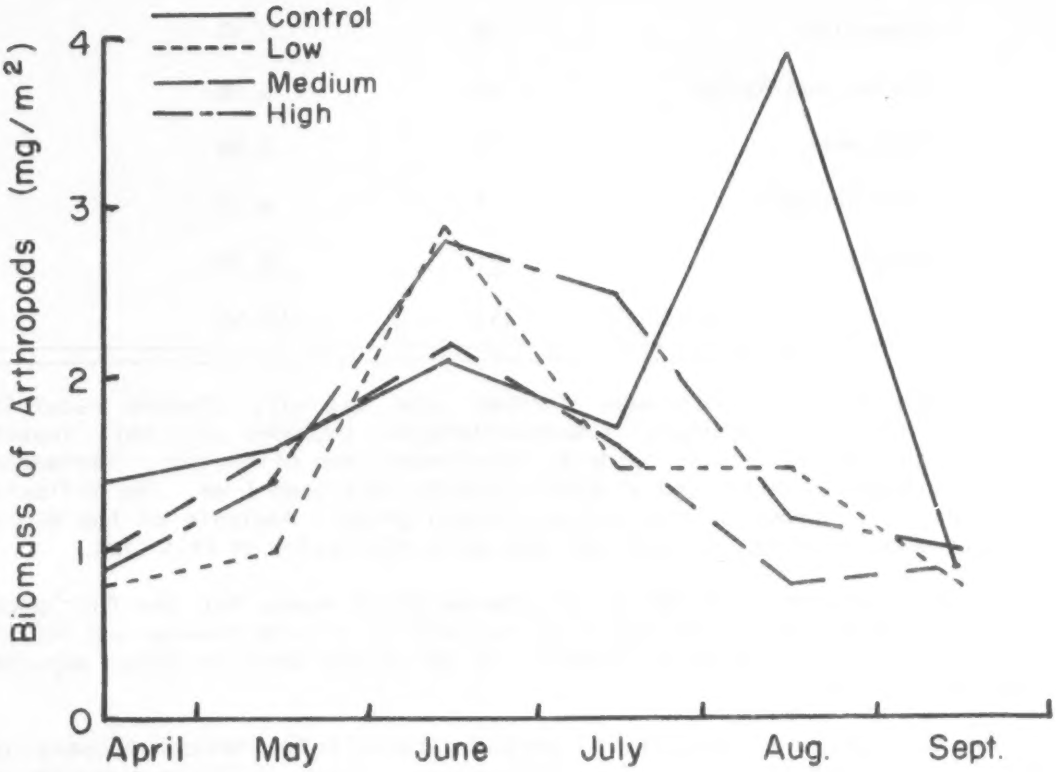

Figure 10.14. Seasonal changes in aboveground arthropod biomass (excluding ants), Taylor Creek, 1975.

TABLE 10.6. TROPHIC DISTRIBUTION OF ARTHROPODS, TAYLOR CREEK, 1975.

| Arthropods | No. of Species | % of Total |
|---|---|---|
| Tissue feeders | 63 | 25.10 |
| Sap feeders | 52 | 20.72 |
| Predators | 47 | 18.73 |
| Scavengers | 20 | 7.97 |
| Parasites | 18 | 7.17 |
| Pollen and nectar | 13 | 5.18 |
| Omnivores | 9 | 3.58 |
| Seed feeders | 1 | 0.40 |
| Others | 27 | 10.75 |
| Total | 250 | 100.00 |

Belowground arthropods were divided into two size classes requiring different sampling techniques. Macroarthropods included all soil insects greater than 1 mm as well as spiders, centipedes, and millipedes, Microarthropods included the mites and wingless insects less than 1 mm. The following discussion is confined to the macroarthropod group. Analysis of the microarthropod data is incomplete and does not merit discussion at this time.

Total macroarthropods based on time-weighted means for the four post-fumigation sample dates indicated high variability of both biomass and numbers among treatments. There were, however, a few groups that inhibited apparent treatment response.

The Coleoptera (Figure 10.15) showed substantially reduced biomass and numbers in the high treatment. Grasshopper eggs (Figure 10.15) were reduced in numbers and biomass on all of the fumigated treatments, most likely the result of avoidance by ovipositing females.

Predators (Figure 10.16) were the only trophic group to show a strong treatment response and this was particularly evident in the predaceous Carabidae (Figure 10.16) with a three-fold difference in both biomass and numbers between the CONTROL and HIGH concentration treatment.

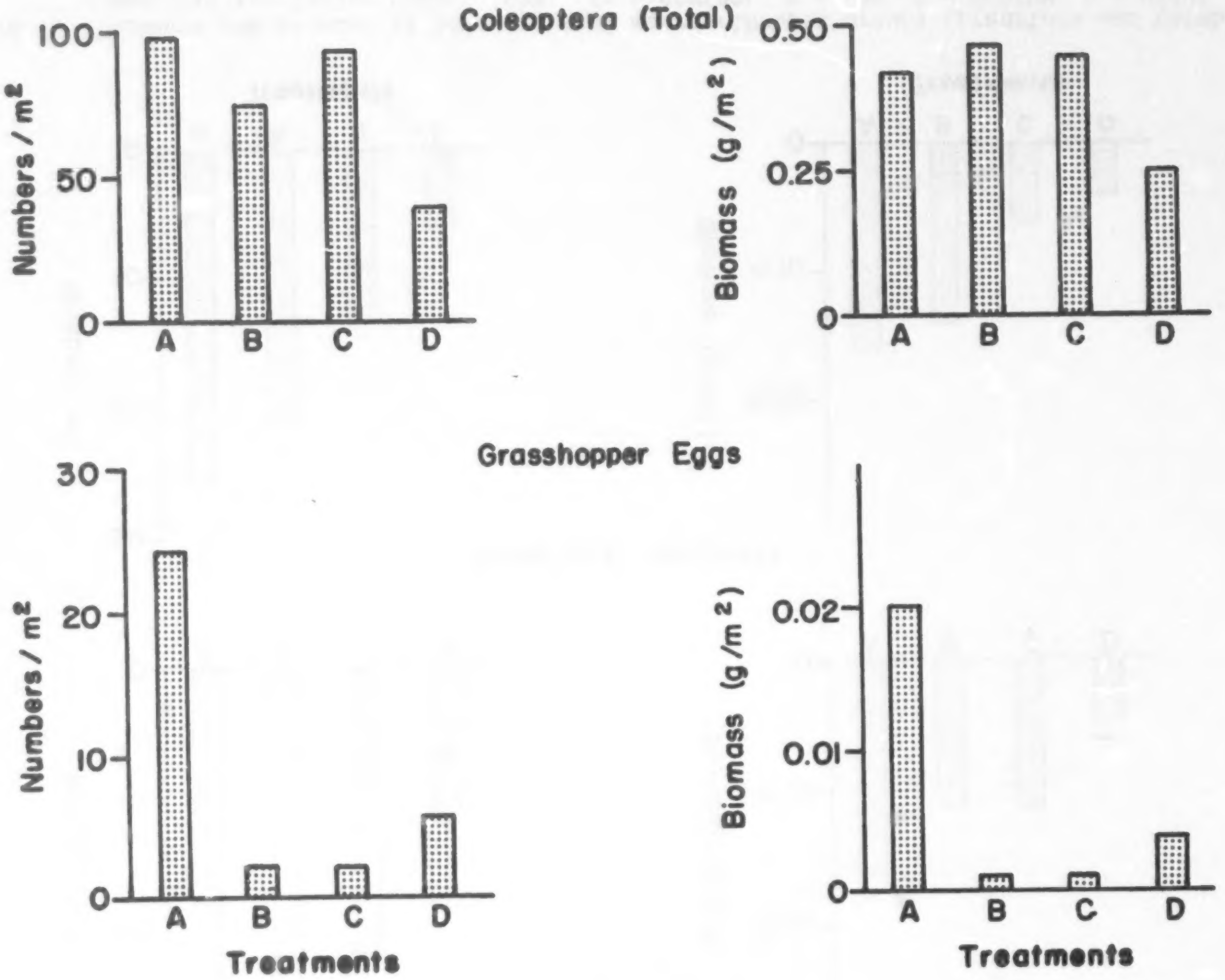

Figure 10.15. Numbers and biomass of selected soil macroarthropod groups (total Coleoptera and grasshopper eggs) for Taylor Creek, 1975. (A = CONTROL, B = LOW, C = MEDIUM, D = HIGH.)

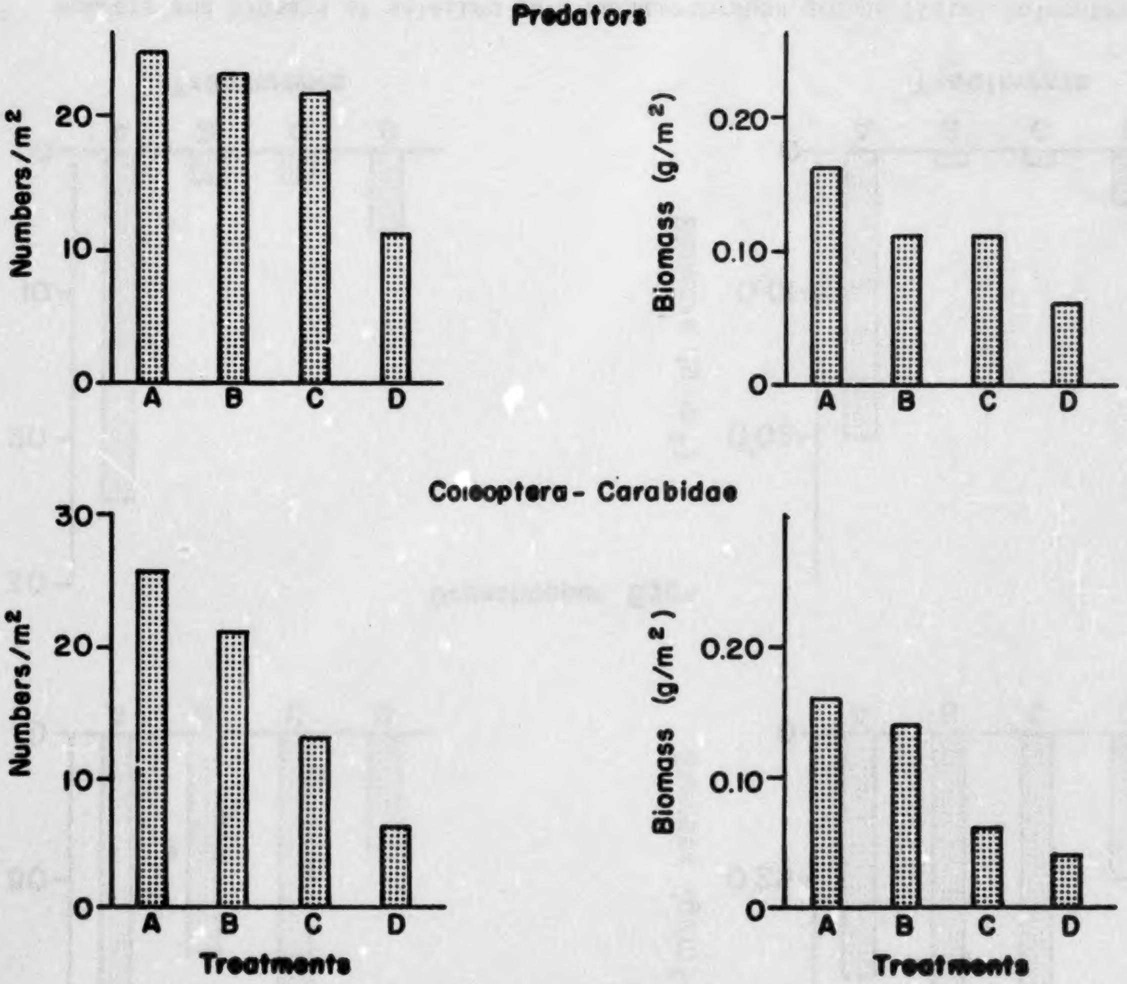

Figure 10.16. Numbers and biomass of selected soil macroarthropod groups (predators and carabid beetles) for Taylor Creek, 1975. (A = CONTROL, B = LOW, C = MEDIUM, D = HIGH)

## SUMMARY

In summary, continuous fumigation of a mixed prairie grassland during the growing season has resulted in only subtle effects on primary producers and arthropod consumers. Although the northern mixed-grass prairie may be particularly resistant to sulfur dioxide fumigation, there are several reasons why substantial changes were not recorded: the most important of these is that fumigation was not begun until 1 June which was approximately 2 months after the beginning of the growing season. These experiments are being continued this year and fumigation was started on 10 April. These data will help explain the 1975 results.

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## APPENDIX

### PHENOLOGY OF MAJOR GRASS SPECIES, TAYLOR CREEK STUDY SITES, 1975
(A = CONTROL, B = LOW, C = MEDIUM, D = HIGH)

| Date | *Poa secunda* | | | | *Stipa comata* | | | | *Bromus japonicus* | | | | *Koeleria cristata* | | | | *Aristida longiseta* | | | | *Bouteloua gracilis* | | | | *Agropyron smithii* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D |
| 27 May | 6 | 6 | 6 | 6 | | 5 | | | | | | | 7 | 7 | 7 | 7 | 2 | 2 | 2 | 2 | | | | | 4 | 4 | 4 | 4 |
| 3 Jun | 8 | 8 | 8 | 8 | | 6 | 6 | 7 | | | 7 | | 8 | 8 | 8 | 8 | 3 | 3 | 3 | 3 | | | | | 4 | 4 | 4 | 4 |
| 10 Jun | 8 | 8 | 8 | 8 | 7 | 7 | 7 | 7 | 7 | 5 | 7 | 7 | 8 | 8 | 8 | 8 | 3 | 3 | 4 | 4 | | | | | 4 | 4 | 4 | 4 |
| 16 Jun | 9 | 9 | 8 | 8 | 7 | 7 | 7 | | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 4 | 4 | 7 | 7 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| 22 Jun | 9 | 9 | 9 | 9 | 7 | 7 | 7 | 7 | 8 | 8 | 8 | 8 | 8 | 9 | 9 | 9 | 4 | 4 | 7 | 7 | 4 | 4 | 4 | 4 | 7 | 7 | 7 | 7 |
| 30 Jun | 9 | 10 | 9 | 10 | 9 | 9 | 9 | 10 | 8 | 8 | 8 | 10 | 9 | 9 | 9 | 10 | 7 | 7 | 7 | 7 | 5 | 5 | 5 | 5 | 7 | 7 | 7 | 7 |
| 7 Jul | 11 | 11 | 11 | 11 | 10 | 10 | 10 | 10 | 9 | 9 | 9 | 10 | 11 | 11 | 11 | 11 | 7 | 7 | 7 | 7 | 5 | 5 | 5 | 5 | 8 | 8 | 8 | 8 |
| 13 Jul | 13 | 13 | 13 | 13 | 12 | 13 | 12 | 12 | 9 | 9 | 9 | 10 | 12 | 12 | 12 | 12 | 8 | 8 | 8 | 8 | 6 | 6 | 6 | 6 | 9 | 9 | 9 | 9 |
| 21 Jul | 14 | 14 | 13 | 14 | 13 | 13 | 14 | 13 | 11 | 11 | 11 | 11 | 13 | 13 | 13 | 13 | 8 | 8 | 8 | 8 | 6 | 6 | 6 | 6 | 9 | 9 | 9 | 9 |
| 30 Jul | | | 14 | | 13 | 14 | | 14 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 12 | 11 | 11 | 11 | | 9 | 9 | 9 | 10 | 10 | 10 | 10 |
| 4 Aug | | | | | 14 | | | | 14 | 14 | 14 | 14 | 13 | 13 | 13 | 13 | 12 | 12 | 12 | 12 | 10 | 10 | 10 | 10 | 12 | 12 | 12 | 12 |
| 11 Aug | | | | | | | | | | | | | 13 | 13 | 13 | 13 | 12 | 13 | 13 | 13 | 10 | 10 | 10 | 10 | 12 | 12 | 12 | 12 |
| 18 Aug | | | | | | | | | | | | | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 11 | 11 | 11 | 11 | 12 | 12 | 12 | 12 |
| 26 Aug | | | | | | | | | | | | | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 |
| 2 Sep | | | | | | | | | | | | | | | | | | | | | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 |
| 10 Sep | | | | | | | | | | | | | | | | | | | | | 14 | 14 | 14 | 14 | 13 | 13 | 13 | 13 |
| 15 Sep | | | | | | | | | | | | | | | | | | | | | | | | | 13 | 13 | 13 | 13 |

(continued)

APPENDIX (continued).

| Date | Taraxacum officinale | | | | Antennaria rosea | | | | Tragopogon dubius | | | | Achillea millifolium | | | | Psoralea argophylla | | | | Sphaeralcea coccinea | | | | Artemisia frigida | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D |
| 27 May | 10 | 10 | 10 | 10 | 8 | 8 | 8 | 8 | 5 | 5 | 5 | 5 | | | | | | | | | | | | | | | | |
| 3 Jun | 12 | 12 | 12 | 12 | 8 | 8 | 8 | 8 | 7 | 7 | 7 | 7 | | | | | | 2 | 2 | | | | | | | | | |
| 10 Jun | 12 | 12 | 12 | 12 | 9 | 9 | 9 | 9 | 7 | 8 | 7 | 8 | | | | | 3 | 3 | 3 | | 4 | 4 | 7 | 4 | | | | |
| 16 Jun | 12 | 12 | 12 | 12 | 9 | 9 | 9 | 9 | 8 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 3 | 3 | 4 | 3 | 4 | 4 | 7 | 7 | | 4 | 4 | 5 |
| 22 Jun | 12 | 12 | 12 | 14 | 9 | 9 | 9 | 9 | 8 | 9 | 9 | 9 | 9 | 9 | 9 | | 3 | 4 | 4 | 3 | 7 | 4 | 7 | 7 | 5 | 5 | 5 | 5 |
| 30 Jun | 12 | 13 | 14 | | 13 | 13 | 13 | 13 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 7 | 7 | 7 | 9 | 9 | 9 | 9 | 9 | 5 | 5 | 5 | 5 |
| 7 Jul | 14 | 14 | | | 13 | 13 | 13 | 13 | 12 | 12 | 12 | 12 | 10 | 10 | 10 | 10 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 5 | 5 | 5 | 5 |
| 13 Jul | | | | | 13 | 13 | 13 | 13 | 12 | 12 | 12 | 12 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 6 | 6 | 6 | 6 |
| 21 Jul | | | | | 14 | 14 | 14 | 14 | 12 | 12 | 13 | 13 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 6 | 6 | 6 | 6 |
| 30 Jul | | | | | | | | | 13 | 13 | 13 | 14 | 11 | 11 | 11 | 11 | 10 | 10 | 10 | 10 | 12 | 12 | 12 | 12 | 6 | 6 | 6 | 6 |
| 4 Aug | | | | | | | | | 14 | 14 | 14 | | 13 | 13 | 13 | 13 | 12 | 12 | 12 | 12 | 13 | 13 | 13 | 13 | 7 | | 6 | 6 |
| 11 Aug | | | | | | | | | | | | | 14 | 14 | 14 | 14 | 12 | 12 | 12 | 12 | 13 | 13 | 13 | 13 | 7 | | 7 | 7 |
| 18 Aug | | | | | | | | | | | | | | | | | 12 | 12 | 12 | 12 | 13 | 13 | 13 | 13 | 8 | | 8 | 8 |
| 26 Aug | | | | | | | | | | | | | | | | | 12 | 12 | 12 | 12 | 13 | 13 | 13 | 13 | 8 | 8 | 8 | 8 |
| 2 Sep | | | | | | | | | | | | | | | | | 12 | 13 | 13 | 13 | 13 | 13 | 13 | | 9 | 9 | 9 | 8 |
| 10 Sep | | | | | | | | | | | | | | | | | 12 | 14 | 14 | 14 | 13 | 13 | 13 | | 9 | 9 | 9 | 9 |
| 15 Sep | | | | | | | | | | | | | | | | | 14 | | | | 13 | 13 | 13 | | 10 | 10 | 10 | 10 |

# SECTION 11

# MONITORING PLANT COMMUNITY CHANGES DUE TO $SO_2$ EXPOSURE

by

J. E. Taylor and W. C. Leininger

## INTRODUCTION

This section describes how the plant community monitoring procedures discussed in Section 2 have been used to monitor changes occurring on the Zonal Air Pollution System (ZAPS) where known levels of sulfur dioxide ($SO_2$) exposure have occurred.

## DISCUSSION

Earlier studies (Taylor et al., 1975, 1976) have shown that Shannon-Weaver and Simpson's indices worked best in the Colstrip project situation. Other indices (Redundancy, Probability of Inter- and Intra-Specific Encounters) were used in 1975, but appeared to be insensitive or inconsistent in response to conditions in this northern Great Plains ecosystem. The Shannon-Weaver indices from ZAPS I were tested for statistical significance. Results are shown in Table 11.1 and Figure 11.1.

The growing season conditions in 1975 were reflected in the diversity indices. The spring was unusually cold, which delayed plant growth. This is reflected in the relatively low indices observed in June and in the generally lower values in 1975 compared with 1974. By July, diversity indices tended to be higher, reflecting the abundant and diverse plant growth once growing conditions became favorable. (There was good summer moisture). This was not the case in the control plots. There was no significant difference among Shannon-Weaver or Simpson's Indices for the control plots among sampling periods. However, the indices associated with $SO_2$ application rates all significantly increased in July and decreased in September. At the latter date, all were significantly ($P \leq .001$) lower than those of the control.

At the July sampling period, the $SO_2$ plots showed diversity indices which increased with their rates of gas application. This may be due to an enhancement of plant growth in the period of early fumigation. By the September date, however, the exact opposite was observed. All three $SO_2$ plots were significantly ($P \leq .001$) less diverse than the control. Both indices showed this effect. This appears consistent with the observations reported in the remote sensing section (Section 7).

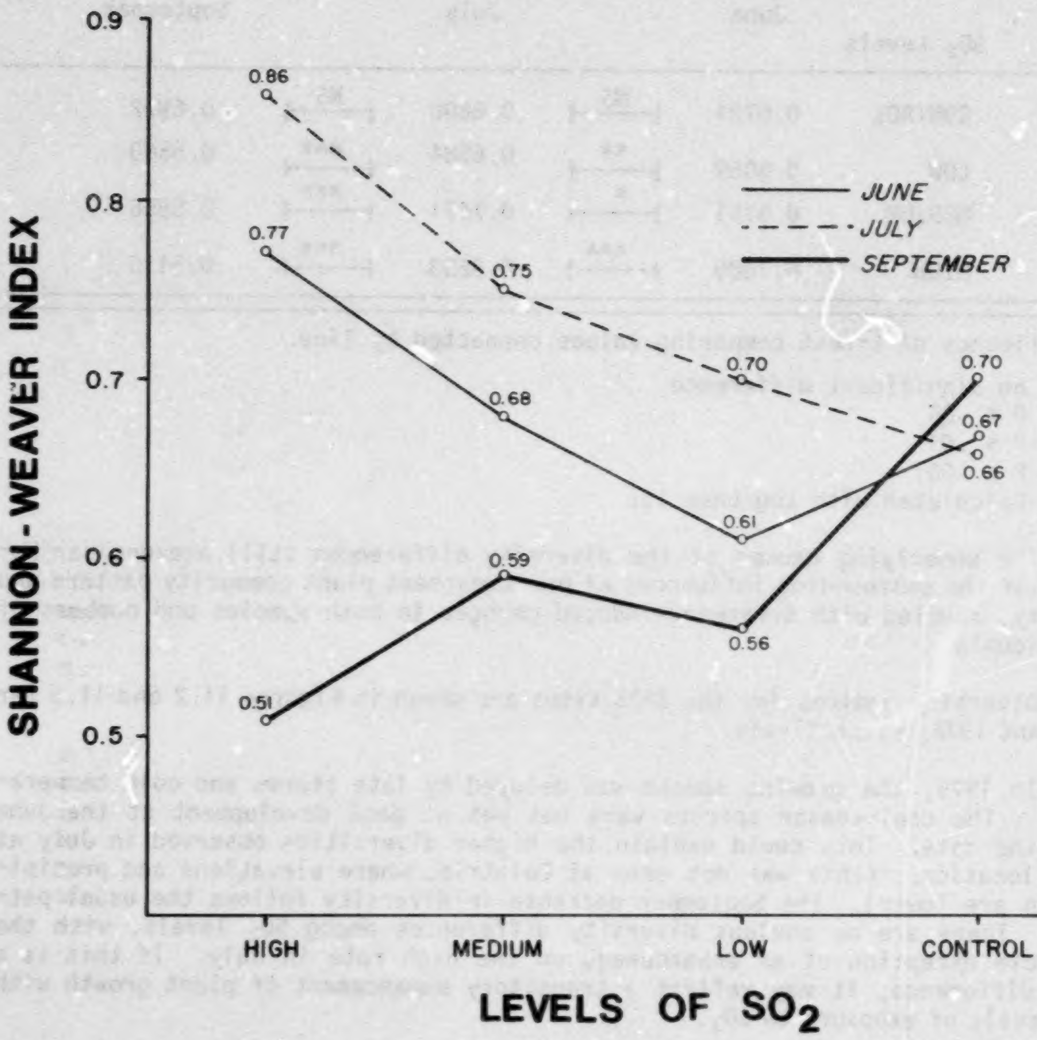

Figure 11.1. Shannon-Weaver index of diversity values for four $SO_2$ levels over three sampling periods, 1975.

TABLE 11.1. STATISTICAL SIGNIFICANCE OF SHANNON-WEAVER INDICES[1] AMONG ZAPS PLOTS, 1975. (Values are averages of 2 lines of plots per observation)

| | SAMPLING PERIODS | | | | |
|---|---|---|---|---|---|
| SO$_2$ Levels | June | | July | | September |
| CONTROL | 0.6724 | NS | 0.6600 | NS | 0.6972 |
| LOW | 0.6059 | ** | 0.6984 | *** | 0.5560 |
| MEDIUM | 0.6761 | * | 0.7471 | *** | 0.5856 |
| HIGH | 0.7689 | *** | 0.8633 | *** | 0.5116 |

Significance of t-test comparing values connected by line.

NS = no significant difference
* = $P \leqq .05$
** = $P \leqq .01$
*** = $P \leqq .001$
[1] = Calculated with Log base 10.

The underlying causes of the diversity differences still are unclear because of the confounding influences of pre-treatment plant community pattern and density, coupled with treatment-induced changes in both species and numbers of individuals.

Diversity indices for the ZAPS sites are shown in Figures 11.2 and 11.3 for 1975 and 1976, respectively.

In 1975, the growing season was delayed by late storms and cold temperatures. The cool-season species were not yet at peak development at the June sampling date. This could explain the higher diversities observed in July at this location. (This was not seen at Colstrip, where elevations and precipitation are lower). The September decrease in diversity follows the usual pattern. There are no obvious diversity differences among SO$_2$ levels, with the possible exception of an enhancement on the high rate in July. If this is a real difference, it may reflect a transitory enhancement of plant growth with low levels of exposure to SO$_2$.

A more typical growing season occurred in 1976. Thus, the seasonal changes in diversity followed the expected trend.

It is obvious that the ZAPS II site is inherently less diverse than ZAPS I. It has lower canopy cover values and fewer species. This is illustrated in the diversity values between the ZAPS locations (Figure 11.3). Also, ZAPS I received more precipitation than ZAPS II in 1976, which could contribute to the difference.

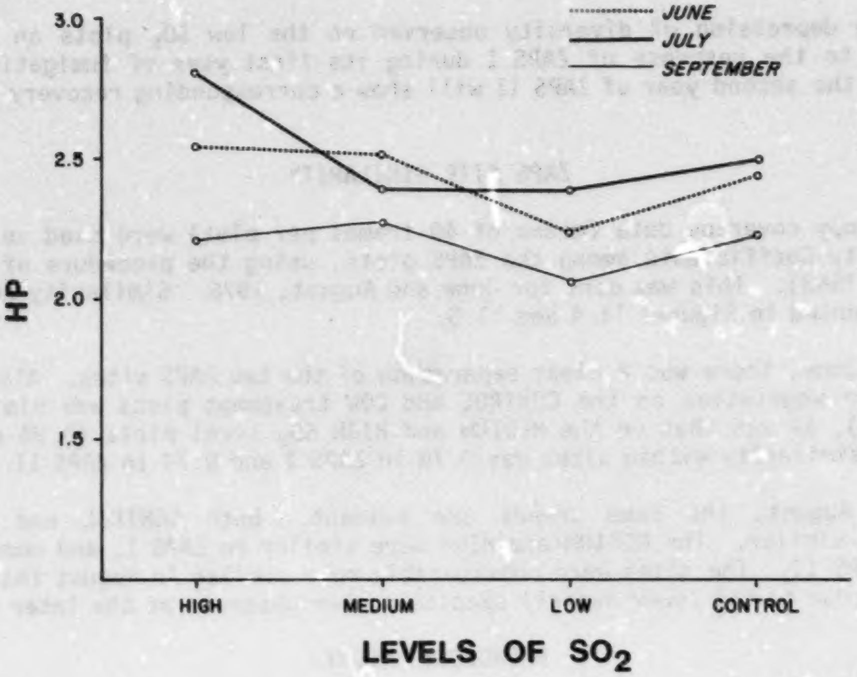

Figure 11.2. Shannon-Weaver function (HP) for ZAPS I, three sampling dates, 1975.

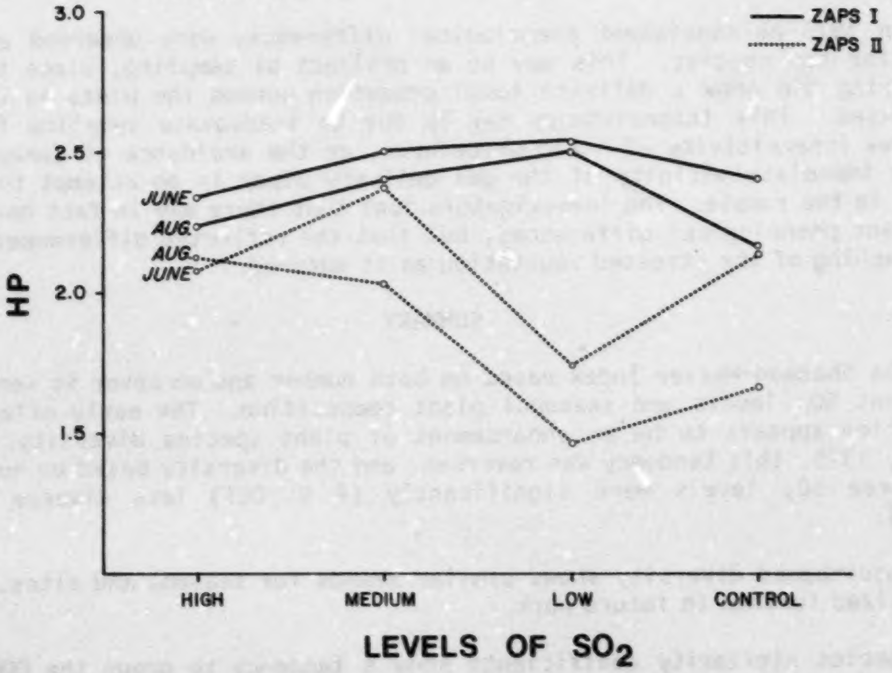

Figure 11.3. Shannon-Weaver function (HP) for ZAPS I and II, two sampling dates each, 1976.

The depression of diversity observed on the low $SO_2$ plots on ZAPS II is similar to the response of ZAPS I during its first year of fumigation (1975). Whether the second year of ZAPS II will show a corresponding recovery remains to be seen.

## ZAPS SITE SIMILARITY

Canopy coverage data (means of 40 frames per plot) were used to calculate Similarity Coefficients among the ZAPS plots, using the procedure of Sokal and Sneath (1963). This was done for June and August, 1976. Similarity dendrograms are presented in Figures 11.4 and 11.5.

In June, there was a clear separation of the two ZAPS sites. Also, in both cases the vegetation on the CONTROL and LOW treatment plots was similar (0.89 and 0.99), as was that on the MEDIUM and HIGH $SO_2$ level plots (0.90 and 0.89). Overall similarity within sites was 0.78 in ZAPS I and 0.71 in ZAPS II.

In August, the same trends are evident. Both CONTROL and LOW plots remained similar. The MEDIUM and HIGH were similar on ZAPS I, and somewhat less so on ZAPS II. The sites were considerably more similar in August than in June, probably due to the lower overall species number observed at the later date.

## PHENOLOGY STUDIES

Phenological stages occurring on the ZAPS plots are shown graphically in Figures 11.6 and 11.7.

In 1975 no consistent phenological differences were observed among ZAPS plots for any species. This may be an artifact of sampling, since the aerial monitoring did show a definite tonal gradation across the plots as the season progressed. This inconsistency may be due to inadequate sampling frequency, relative insensitivity of field procedures, or the avoidance of damaged plants in the immediate vicinity of the gas delivery pipes in an attempt to minimize "bias" in the sample. The investigators feel that there may in fact have been no important phenological differences, but that the reflected differences were due to bleaching of the stressed vegetation as it matured.

## SUMMARY

The Shannon-Weaver Index based on both number and on cover is sensitive to different $SO_2$ levels and seasonal plant composition. The early effect of $SO_2$ fumigation appears to be an enhancement of plant species diversity. By late season, 1975, this tendency was reversed, and the diversity based on numbers for all three $SO_2$ levels were significantly ($P \leq .001$) less diverse than the control.

Cover-based diversity shows similar trends for seasons and sites, and will be utilized further in future work.

Species similarity coefficients show a tendency to group the CONTROL and LOW versus the MEDIUM and HIGH ZAPS treatments on both sites and both dates.

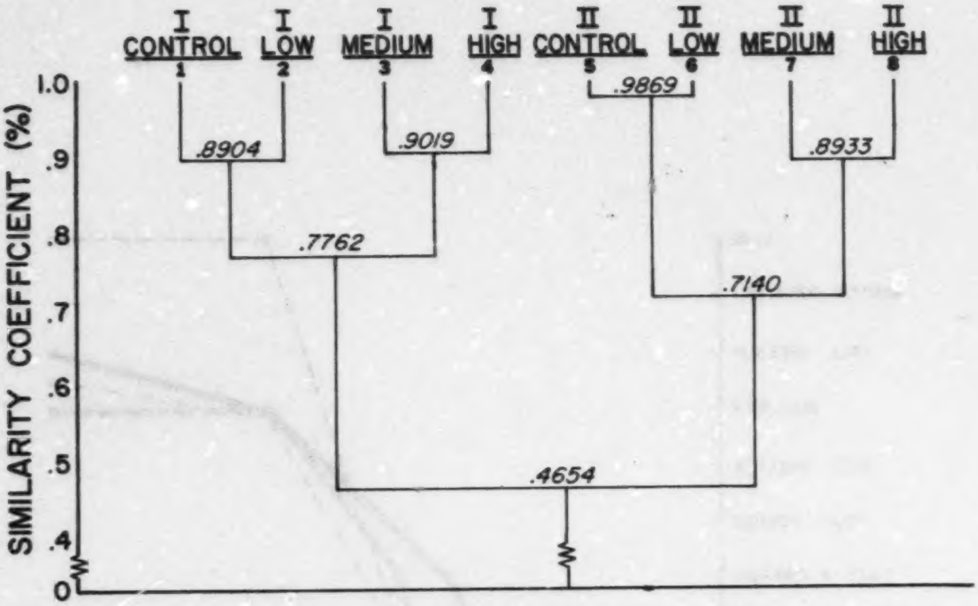

Figure 11.4. Dendrogram of similarity coefficients for the ZAPS sites, based on canopy coverage, August 1976.

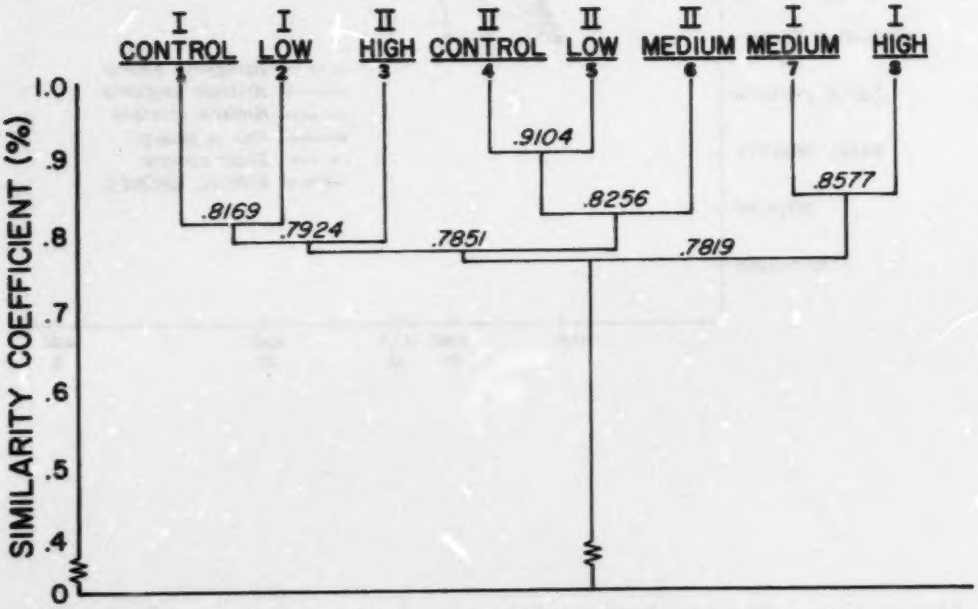

Figure 11.5. Dendrogram of similarity coefficients for the ZAPS sites, based on canopy coverage, June 1976.

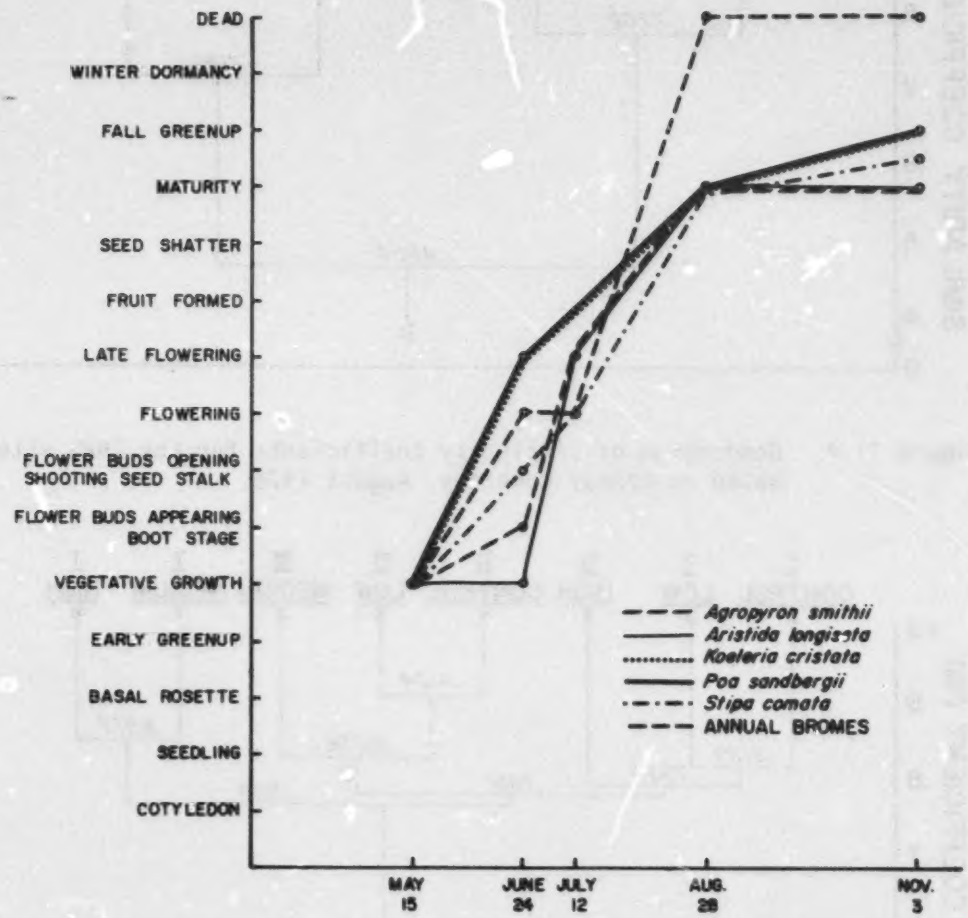

Figure 11.6. Phenological profile of selected species on Taylor Creek control plot.

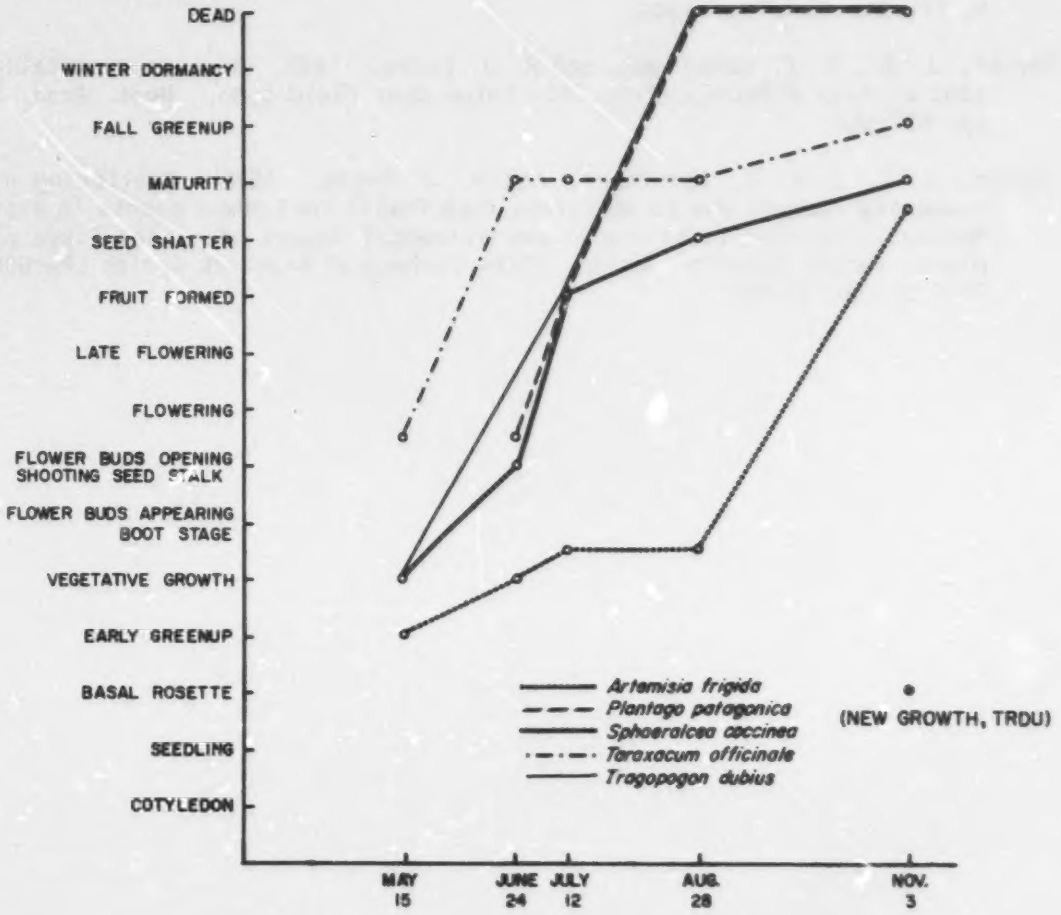

Figure 11.7. Phenological profile of selected species on Taylor Creek control plot.

The phenology scoring system has proved very satisfactory in a variety of vegetation types. However, phenology changes attributable to $SO_2$ have not been quantified because of the many confounding influences. The frequency and intensity of sampling is being increased to construct a more complete data base so that confounding influences can be identified and separated.

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## SECTION 12

# EFFECTS OF LOW-LEVEL $SO_2$ STRESS ON TWO LICHEN SPECIES

by

S. Eversman

## INTRODUCTION

As part of the development of western energy resources, coal-fired power plants are proliferating in the Great Plains region. Two 350-megawatt generating plants began operation in autumn, 1975, and spring, 1976, in Colstrip in southeast Montana. Two more are in the planning stages.

Sulfur dioxide ($SO_2$), one of the emissions from burning coal, has been repeatedly demonstrated to cause adverse effects in plants, especially lichens. Many laboratory experiments have shown chlorophyll bleaching, decreases in respiration and photosynthetic rates, and plasmolysis with $SO_2$ exposure (LeBlanc and Rao, 1975). The lowest experimental $SO_2$ concentration has been at 0.50 ppm for 12 hours (Nash, 1975).

LeBlanc and Rao (1975) cite experimental works that draw these conclusions. Transplanting lichen species into areas already polluted results in lichens exhibiting the same internal and external pathological symptoms demonstrated in laboratory tests. Most of the studies have been in humid areas where $SO_2$ is thought to be more harmful than in semi-arid climates. They estimate that, in humid climates, long-range average concentrations of $SO_2$ below 0.002 ppm would cause no damage to lichen species; acute damage occurs above 0.03 ppm, and intermediate amounts of $SO_2$ would cause chronic damage to lichens.

This report presents results of observations on the effects of chronic low-level $SO_2$ stress on two lichens native to semi-arid grassland and ponderosa pine vegetation types. The objectives of the study were to:

1) Identify and time the anatomical and physiological changes that occurred with exposure to various low levels of $SO_2$, and

2) to make simultaneous observations on associated vascular vegetation for comparison purposes.

_Usnea hirta_ is an epiphyte on trunks and branches of ponderosa pine, the major tree of this region It is a fruticose lichen, i.e., stringy in appearance; this type of lichen is considered particularly susceptible and sensitive to air pollutants.

Parmelia chlorochroa is a foliose (leafy, flat) lichen that lives on bare soil between grass clumps and shrubs on the grasslands. Foliose lichens are also considered sensitive to air pollution.

A fumigation system was constructed on a grassland site in southeast Montana. The system was composed of 4 plots, each about 0.654 hectare. Each plot had a network of aluminum pipes that delivered different amounts of $SO_2$ through small holes in the bottoms of the pipes. Lee and Lewis (1976) fully describe the system. One system of four plots was in operation in 1975 and 1976, and a duplicate system operated in 1976 about 1 km from the first site. This report refers to the system operating in both 1975 and 1976 as ZAPS I (I = Site 1, ZAPS = Zonal Air Pollution System). The fumigation or treatment plots, within each site or system, are referred to as A (CONTROL), B (LOW), C (MEDIUM) and D (HIGH) (Lee and Lewis, 1976). ZAPS II refers to the second system, with similar treatment plots A, B, C and D, operating in 1976.

By specifically identifying the characteristics of the two lichen species exposed to the $SO_2$ stress on the ZAPS sites, these species can be used as biological systems to monitor increasing $SO_2$ content of the air in the Colstrip area. Simultaneous observations made by other researchers in the region will be combined with the lichen observations to identify and interrelate ecosystem components affected by decreasing air quality.

Usnea hirta and Parmelia chlorochroa from several ponderosa pine and grassland field study sites have been collected and observed for baseline conditions. They have been compared with the stressed lichens from the ZAPS sites. The sites are mapped and described in a previous chapter (Eversman, 1976).

## METHODS

Five metal fenceposts were placed nonrandomly in each of the ZAPS fumigation plots. They were placed toward each corner and in the center of each plot where there was minimum grass cover so that Parmelia chlorochroa could be transplanted to the soil. There was a minimum of 2 meters between a fencepost and any fumigation pipe.

Transplants of both Usnea hirta and Parmelia chlorochroa were made 1 May 1975 and 23 March 1976; fumigations began 24 May 1975 and 12 April 1976. Ponderosa pine branches containing U. hirta were wired onto the northeast side of each fencepost so the branches extended about 0.25 - 1.0 meters vertically on the post. P. chlorochroa was moved from the soil in a nearby field (Site P7) to the fumigation sites to augment the existing populations. A second set of P. chlorochroa transplants was made 17 July 1976 from Site G5 (Kluver East) to compare vertical position effects of the $SO_2$ stress. P. chlorochroa samples were tied on bare ponderosa pine branches and wired to the fenceposts on Site 1, parallel to the branches containing U. hirta. Duplicate samples were placed on the soil at the base of each fencepost.

Samples were collected about once a month through September of each year. They were observed for general thallus condition and photographed. Respiration rates were determined manometrically (Eversman, 1975). Samples were sent to the Montana State University Soil Testing Laboratory for sulfur analysis. The details of analytical methods are presented in the Appendix.

Plasmolysis counts were made by preparing 3 wet-mount slides of 3 different plants and counting 100 algal cells on each slide, recording the number of plasmolyzed cells.

The same set of observations was made on lichen samples collected at about the same times, 20-97 km from the fumigation sites.

Stems and leaves of Agropyron smithii and Koeleria cristata, two grasses, were collected near each fencepost on Sites 1 and 2 for 3 months in 1976, to determine respiration rates and sulfur content.

## RESULTS

Respiration rates and results of the completed plasmolysis counts for Usnea hirta appear in Table 12.1. Samples from the A (CONTROL) plot on both Sites 1 and 2 did not exhibit respiration rate and plasmolysis percentages different from those of U. hirta samples collected simultaneously from their natural habitats.

The respiration rates of samples from Plot B were consistently below those of samples from the control site, but within the range of samples from all unpolluted sites. The plasmolysis level reached about 76% of the control levels in 100 days, and leveled off throughout the 156 days of $SO_2$ treatment. Plasmolyzed algal cells are also bleached.

The respiration rates of U. hirta samples from plot C decreased to 57% of those of control plot A samples, and remained at less than 50% of control rates for the remainder of the test period. Plasmolysis was nearly 100% in 31-33 days, and the algae never recovered.

Samples from plot D exhibited a respiration rate that was 6% of that of control samples in 1975, but in 1976 the rates fell to 33-46% of control plot A samples. They remained 19-36% of those of control samples for the test period. Plasmolysis and bleaching reached 100% in 31-33 days, with no recovery.

P. chlorochroa samples on the ground did not exhibit changes in respiration rates in any of the treatment plots (Table 12.2). The results of plasmolysis counts are still very incomplete, but some representative counts showed a slight increase of plasmolysis with $SO_2$ stress. When samples were elevated to the fenceposts with the U. hirta, however, plasmolysis increased to 96% in 23 days on plot D, and the mean respiration rate dropped to 22% of that of control (A) samples. Respiration rates of elevated P. chlorochroa decreased on plots B, C, and D; the rates of control samples, A, and those on the ground on all plots, did not decrease.

Grass samples exhibited a generally decreasing respiration rate on all plots from June to August (Table 12.3). This decrease does not superficially appear to be related to $SO_2$ dosage.

TABLE 12.1. RESPIRATION RATES AND PERCENTAGE OF PLASMOLYSIS FOR *Usnea hirta* ON ZAPS SITES AND ON SOME COLSTRIP FIELD SITES, 1975-76. Respiration rates are given as the mean of 3-10 samples ± 1 standard deviation, expressed in µl $O_2$ consumed/g dry weight/hour. Plasmolysis is expressed as mean percentage of 3 samples ± 1 standard deviation.

| Collection Site | Number of $SO_2$ Treatment Days, | Date | Resp. Rate $\bar{x}$ | 1 s.d. | Plasm. % $\bar{x}$ | 1 s.d. |
|---|---|---|---|---|---|---|
| P10, East Otter Creek | 0 | 3-23-76 | 709 | 63 | 3 | 3 |
| P9, transplant source | 0 | 5-01-75 | 563 | 109 | | |
| P15, Fort Howes natives | 0 | " | 511 | 213 | | |
| P15, Fort Howes natives | 0 | 5-13-76 | 576 | 113 | | |
| P16, Poker Jim Butte | 0 | " | 694 | 183 | | |
| ZAPS: IA | 31 | " | 711 | 72 | 6 | 1 |
| IIA | " | " | 800 | 97 | 4 | 3 |
| IB | " | " | 718 | 141 | 35 | 25 |
| IIB | " | " | 755 | 74 | 37 | 39 |
| IC | " | " | 290 | 155 | 94 | 9 |
| IIC | " | " | 438 | 175 | 99 | 2 |
| ID | " | " | 325 | 199 | 94 | 9 |
| IID | " | " | 264 | 99 | 100 | 0 |
| P6, Kluver West tr. | 0 | 6-25-75 | 864 | 86 | | |
| ZAPS: IA | 33 | " | 821 | 37 | 6 | 4 |
| IB | " | " | 746 | 98 | | |
| IC | " | " | 472 | 204 | | |
| ID | " | " | 53 | 45 | | |
| P16, Poker Jim Butte | 0 | 7-16-75 | 630 | 77 | | |
| P13, Home Creek Butte | " | " | 462 | 55 | | |
| P11, SEAM 1 | " | " | 545 | 35 | | |
| ZAPS: IA | 47 | 7-10-75 | 686 | 115 | | |
| IB | " | " | 555 | 121 | 54 | 20 |
| IC | " | " | 333 | 307 | 99 | 1 |
| ID | " | " | 0 | 0 | 100 | 0 |
| P10, East Otter Creek | 0 | 6-23-76 | 744 | 45 | 11 | 2 |
| ZAPS: IA | 72 | " | 704 | 71 | 4 | 2 |
| IIA | " | " | 555 | 34 | 4 | 2 |
| IB | " | " | 359 | 78 | 63 | 18 |
| IIB | " | " | 476 | 77 | 58 | 21 |
| IC | " | " | 236 | 83 | 100 | 1 |
| IIC | " | " | 263 | 48 | 99 | 1 |
| ID | " | " | 115 | 67 | 100 | 1 |
| IID | " | " | 169 | 38 | 100 | 1 |
| P9, Road | 0 | 8-11-75 | 508 | 50 | | |

(continued)

TABLE 12.1 (continued)

| Collection Site | Number of SO$_2$ Treatment Days, | Date | Resp. Rate $\bar{x}$ | 1 s.d. | Plasm. % $\bar{x}$ | 1 s.d. |
|---|---|---|---|---|---|---|
| ZAPS: IA | 79 | " | 738 | 66 | | |
| IB | " | " | 435 | 87 | | |
| IC | " | " | 316 | 133 | | |
| ID | " | " | 0 | 0 | | |
| P10, East Otter Creek | 0 | 7-15-76 | 463 | 57 | 9 | 3 |
| P16, Poker Jim Butte | " | " | 610 | 31 | | |
| P11, SEAM 1 | " | " | 453 | 19 | | |
| ZAPS: IA | 96 | 7-17-76 | 670 | 103 | 5 | 2 |
| IIA | " | " | 643 | 38 | 5 | 2 |
| IB | " | " | 473 | 39 | 69 | 17 |
| IIB | " | " | 479 | 86 | 66 | 18 |
| IC | " | " | 323 | 35 | 99 | 2 |
| IIC | " | " | 264 | 49 | 99 | 2 |
| ID | " | " | 244 | 92 | 100 | 0 |
| IID | " | " | 98 | 20 | 100 | 0 |
| P10, East Otter Creek | 0 | 9-25-75 | 640 | 40 | | |
| P8, Morning Star View | " | " | 828 | 135 | | |
| P3, Kluver NE1 | " | " | 736 | 72 | | |
| ZAPS: IA | 110 | 9-11-75 | 709 | 55 | 15 | 3 |
| IB | " | " | 499 | 128 | 77 | 16 |
| IC | " | " | 355 | 137 | 98 | 3 |
| ID | " | " | 0 | 0 | 100 | 0 |
| P10, East Otter Creek | 0 | 8-09-75 | 668 | 41 | | |
| P14, 3-Mile top | " | " | 667 | 76 | | |
| P14, 3-Mile bottom | " | " | 547 | 40 | | |
| ZAPS: IA | 119 | " | 721 | 18 | 5 | 2 |
| IIA | " | " | 618 | 50 | 24 | 10 |
| IB | " | " | 556 | 84 | | |
| IIB | " | " | 509 | 66 | | |
| IC | " | " | 298 | 73 | | |
| IIC | " | " | 282 | 84 | | |
| ID | " | " | 150 | 29 | | |
| IID | " | " | 118 | 16 | | |
| P10, East Otter Creek | 0 | 9-15-76 | 633 | 53 | 10 | 1 |
| P15, Fort Howes transplants | " | " | 597 | 43 | | |
| P15, Fort Howes natives | " | " | 470 | 50 | | |
| ZAPS: IA | 156 | 9-14-76 | 631 | 21 | 20 | 5 |
| IIA | " | " | 506 | 61 | 4 | 2 |
| IB | " | " | 487 | 43 | 82 | 13 |
| IIB | " | " | 457 | 37 | 71 | 18 |
| IC | " | " | 340 | 40 | 100 | 1 |
| IIC | " | " | 289 | 85 | 99 | 1 |
| ID | " | " | 142 | 68 | 100 | 0 |
| IID | " | " | 112 | 46 | 100 | 0 |

TABLE 12.2. RESPIRATION RATES AND PERCENTAGE OF PLASMOLYSIS FOR Parmelia chlorochroa ON SOME COLSTRIP FIELD SITES, 1975-1976. Respiration rates are given as the mean of 3-10 samples ± 1 standard deviation, expressed in µl $O_2$ consumed/g dry weight/hour. Plasmolysis is expressed as mean percentage of 3 samples ± 1 s.d.

| Collection Site | Number of $SO_2$ Treatment Days, | Date | Resp. Rate $\bar{x}$ | 1 s.d. | Plasm. % $\bar{x}$ | 1 s.d. |
|---|---|---|---|---|---|---|
| Field near ZAPS sites | 0 | 5-01-75 | 223 | 39 | | |
| G7, Pasture | " | " | 337 | 132 | | |
| G1, Hay Coulee | 0 | 5-14-76 | 458 | 43 | | |
| G7, Pasture | " | " | 371 | 54 | | |
| ZAPS: IB | 31 | " | 276 | 50 | | |
| IC | " | " | 290 | 15 | | |
| G5, Kluver East | 0 | 6-26-75 | 316 | 40 | | |
| G1, Hay Coulee | " | " | 215 | 26 | | |
| ZAPS: IA | 33 | " | 319 | 44 | 2 | 2 |
| IB | " | " | 330 | 36 | | |
| IC | " | " | 345 | 36 | 8 | 2 |
| ID | " | " | 296 | 54 | | |
| G4, Kluver North | 0 | 7-07-75 | 349 | 21 | | |
| G3, Kluver West | " | " | 293 | 29 | | |
| ZAPS: IA | 47 | 7-10-75 | 345 | 80 | 10 | 7 |
| IB | " | " | 404 | 54 | | |
| IC | " | " | 317 | 50 | 4 | 4 |
| ID | " | " | 294 | 38 | 43 | 34 |
| Field near ZAPS site | 0 | 8-11-75 | 217 | 28 | | |
| ZAPS: IA | 79 | " | 356 | 48 | | |
| IB | " | " | 357 | 36 | | |
| IC | " | " | 336 | 47 | | |
| ID | " | " | 309 | 37 | | |
| G1, Hay Coulee | 0 | 7-14-76 | 299 | 22 | | |
| G3, Kluver West | " | " | 272 | 25 | | |
| G4, Kluver North | " | " | 242 | 4 | | |
| G5, Kluver East | " | " | 322 | 26 | | |
| ZAPS: IA (native) | 96 | 7-17-76 | 231 | 41 | 22 | 3 |
| IIA (native) | " | " | 293 | 36 | | |
| IB | " | " | 299 | 22 | | |
| IIB | " | " | 262 | 31 | | |
| IIC | " | " | 301 | 30 | | |
| IID | " | " | 335 | 22 | | |

(continued)

TABLE 12.2. (continued)

| Collection Site | Number of $SO_2$ Treatments Days, | Date | Resp. Rate x | 1 s.d. | Plasm. % x | 1 s.d. |
|---|---|---|---|---|---|---|
| ZAPS: IA | 110 | 9-11-75 | 362 | 69 | | |
| IB | " | " | 271 | 20 | | |
| IC | " | " | 353 | 52 | | |
| ID | " | " | 340 | 21 | | |
| ZAPS: IA (native) | 119 | 8-09-76 | 350 | 20 | | |
| IID | " | " | 293 | 50 | | |
| After new transplants were made 7-17-76 (* = elevated samples): | | | | | | |
| ZAPS: IA | 23 | 8-09-76 | 298 | 8 | | |
| IIA | " | " | 297 | 28 | | |
| IA* | " | " | 366 | 26 | | |
| ZAPS: IB | " | " | 314 | 25 | | |
| IIB | " | " | 315 | 40 | | |
| IB* | " | " | 311 | 33 | | |
| ZAPS: IC | " | " | 309 | 26 | | |
| IIC | " | " | 368 | 30 | | |
| IC* | " | " | 197 | 54 | | |
| ZAPS: ID | " | " | 396 | 24 | 15 | 6 |
| IID | " | " | 437 | 38 | | |
| ID* | " | " | 79 | 15 | 96 | 3 |
| G1, Hay Coulee | 0 | 9-14-76 | 274 | 22 | | |
| G3, Kluver West | " | " | 301 | 15 | | |
| G4, Kluver North | " | " | 262 | 34 | | |
| G5, Kluver East | " | " | 239 | 15 | | |
| ZAPS: IA (native) | 60 | 9-15-76 | 315 | 31 | | |
| IA | " | " | 305 | 44 | | |
| IIA | " | " | 326 | 31 | | |
| IA* | " | " | 345 | 9 | | |
| ZAPS: IB | " | " | 270 | 33 | | |
| IIB | " | " | 338 | 28 | | |
| IB* | " | " | 174 | 47 | | |
| ZAPS: IC | " | " | 322 | 38 | | |
| IIC | " | " | 403 | 31 | | |
| IC* | " | " | 45 | 10 | | |
| ZAPS: ID | " | " | 396 | 13 | | |
| IID | " | " | 386 | 77 | | |
| ID* | " | " | 43 | 34 | | |

TABLE 12.3. RESPIRATION RATES OF TWO GRASSES, *Koeleria cristata* AND *Agropyron smithii*, FROM THE ZAPS SITES, 1976. Respiration rates are given as the mean of 3 samples ± 1 standard deviation, expressed in µl $O_2$ consumed/g dry weight/hour.

| | | | *Koeleria cristata* | | *Agropyron smithii* | |
|---|---|---|---|---|---|---|
| Collection Plot | Number of $SO_2$ Treatment Days, | Date | Resp. Rate $\bar{x}$ | 1 s.d. | Resp. Rate $\bar{x}$ | 1 s.d. |
| IA | 72 | 6-23-76 | 702 | 51 | 646 | 189 |
| IIA | " | " | 706 | 103 | 579 | 30 |
| IB | " | " | 706 | 102 | 736 | 163 |
| IIB | " | " | 617 | 134 | 699 | 70 |
| IC | " | " | 893 | 104 | 516 | 106 |
| IIC | " | " | 564 | 120 | 551 | 34 |
| ID | " | " | 1045 | 42 | 610 | 235 |
| IID | " | " | 759 | 114 | 349 | 34 |
| IA | 96 | 7-17-76 | 506 | 4 | 355 | 64 |
| IIA | " | " | 247 | 8 | 335 | 38 |
| IB | " | " | 587 | 130 | 231 | 90 |
| IIB | " | " | 411 | 75 | 328 | 120 |
| IC | " | " | 560 | 72 | 197 | 64 |
| IIC | " | " | 430 | 28 | 366 | 163 |
| ID | " | " | 827 | 113 | 212 | 110 |
| IID | " | " | 525 | 103 | 249 | 55 |
| IA | 119 | 8-09-76 | 128 | 35 | 145 | 13 |
| IIA | " | " | 147 | 25 | 312 | 38 |
| IB | " | " | 164 | 19 | 177 | 16 |
| IIB | " | " | 226 | 45 | 107 | 32 |
| IC | " | " | 146 | 29 | 239 | 29 |
| IIC | " | " | 375 | 41 | 394 | 50 |
| ID | " | " | 108 | 31 | 297 | 68 |
| IID | " | " | 332 | 46 | 530 | 85 |

The data from the $SO_4$ content analysis from 1975 (Table 12.4) suggest increasing sulfur content as $SO_2$ exposure continued, but available results are inconclusive. The 1976 analyses are not yet complete.

Microscopic examination revealed extensive bacteria populations associated with the lichens, especially with P. chlorochroa. As $SO_2$ stress increased in dosage and time, the bacteria appeared to be increasingly attached to the fungal hyphae, rather than in the water medium (on a microscope slide). The bacteria were associated less with algae than with the fungal hyphae.

## DISCUSSION

A reasonable question arises in connection with both the Usnea hirta and Parmelia chlorochroa transplants on the control (A) plots. The U. hirta specimens have been transplanted from ponderosa pine sites that are shadier than the fumigation sites and control plot A. However, U. hirta is not a plant of deep shade; it grows on partially exposed branches and trunks of ponderosa pine. Every effort was made to compare the measurements made on U. hirta from control plots A with those of native samples collected directly from their native habitats. The respiration rates, plasmolysis counts, and visual appearance of the U. hirta exhibit no harmful effects from the transplanting.

P. chlorochroa is a native of exposed bare soil and was transplanted in most cases to a similar habitat. Some adverse effects might have been expected when it was tied to pine branches and elevated above the soil, but this was not the case. A common observation when any specimens are transplanted is a slight temporary increase in respiration rate over that of natives or duplicate samples, but the increase is not permanent and no visual effects can be detected.

Decreasing respiration rates, algal bleaching and plasmolysis, and yellowing of the plant U. hirta reflect increasing $SO_2$ exposure.

The respiration rates of P. chlorochroa samples on the ground did not decrease with the $SO_2$ treatments, nor did the plants change color. P. chlorochroa specimens were then placed on the branches 0.75 m above the ground to test if: 1) vertical position in the grassland stratification had any effects and 2) associated bacteria decreased when the thalli were off the ground. The bacterial populations remained visually the same but the respiration rates decreased dramatically in proportion to $SO_2$ dosage, and the percentage of plasmolysis increased to nearly 97% in 23 days on plot D.

This seems to indicate that the $SO_2$ levels on the sites vary appreciably from ground level to 0.5-1.0 m above the ground, although compounding stress effects could be a factor in the observed reactions. Further work must include using some monitoring method, possibly sulfation plates, to detect the vertical distribution of the $SO_2$ on the treatment plots.

Conclusions reached before P. chlorochroa samples were placed on the branches on the test site fenceposts were that U. hirta is a species more sensitive than P. chlorochroa to $SO_2$ stress. Observations of elevated P. chlorochroa samples indicate that both species are sensitive to $SO_2$. However,

TABLE 12.4. SULFATE SULFUR CONTENTS OF Usnea hirta AND Parmelia chlorochroa SAMPLES COLLECTED IN 1975. Expressed as mean percentage ± 1 standard deviation of 3 - 5 samples. Determined by the MSU Soils Testing Laboratory.

| Usnea hirta<br>Collection Site | Number of $SO_2$ Treatment Days, | Date | % Sulfur | 1 s.d. |
|---|---|---|---|---|
| P9, Road | 0 | 5-01-75 | 0.25 | 0.04 |
| P15, Fort Howes tr. | " | " | 0.25 | 0.03 |
| P15, Fort Howes natives | " | " | 0.19 | 0.04 |
| P9, Road | 0 | 8-11-75 | 0.26 | 0.04 |
| P3, Kluver NE1 | 0 | 9-25-75 | 0.03 | 0.01 |
| P8, Morning Star View | 0 | " | 0.19 | 0.08 |
| P13, Home Creek Butte | 0 | 7-16-75 | 0.15 | 0.16 |
| P10, East Otter Creek | " | " | 0.08 | 0.01 |
| " " | 0 | 9-25-75 | 0.22 | 0.11 |
| ZAPS: IA | 47 | 7-10-75 | 0.29 | 0.01 |
| IB | 47 | 7-10-75 | 0.30 | 0.03 |
| IB | 79 | 8-11-75 | 0.17 | 0.15 |
| IB | 110 | 9-11-75 | 0.29 | 0.11 |
| IC | 47 | 7-10-75 | 0.33 | 0.03 |
| IC | 79 | 8-11-75 | 0.19 | 0.11 |
| IC | 110 | 9-11-75 | 0.37 | 0.07 |
| ID | 47 | 7-10-75 | 0.32 | 0.05 |
| ID | 79 | 8-11-75 | 0.41 | 0.01 |
| Parmelia chlorochroa | | | | |
| Pasture near ZAPS sites | 0 | 5-01-75 | 0.17 | 0.02 |
| G7, transplant source | " | " | 0.19 | 0.03 |
| ZAPS: IA | 47 | 7-10-75 | 0.23 | 0.04 |
| IB | 47 | 7-10-75 | 0.19 | 0.02 |
| IB | 79 | 8-11-75 | 0.18 | 0.02 |
| IB | 110 | 9-11-75 | 0.20 | 0.03 |
| IC | 47 | 7-10-75 | 0.23 | 0.03 |
| IC | 110 | 9-11-75 | 0.18 | 0.03 |
| ID | 47 | 7-10-75 | 0.21 | 0.03 |
| ID | 79 | 8-11-75 | 0.22 | 0.01 |

U. hirta may be more useful as an area-wide indicator of decreasing air quality because of its more exposed locations on tree trunks and branches. Wind conditions or filtering by grasses and forbs may prevent a great deal of $SO_2$ from reaching ground level, except in bare areas, where it can demonstrably affect P. chlorochroa.

Simultaneous visual observations were made on the associated vascular vegetation on the fumigation plots. While unmistakable bleaching was evident in U. hirta on all test plots (ZAPS I and II, B, C, and D plots) 31-33 days after the fumigation began, no visual effects appeared on the grasses during the test periods. The height of the grass plants was about equal to the vertical placement of the transplanted lichens. The generally decreasing respiration rates of Agropyron smithii and Koeleria cristata are probably a function of their phenology. The rate of decrease is similar on nearly all plots, both sites, from June to August. Exceptions (samples from 2D and 1C, August, 1976), however, might reflect $SO_2$ stress, which has been demonstrated to raise respiration rates when present in very small amounts (LeBlanc and Rao, 1975).

Other personal observations were leaf-tip curling and browning in Achillea lanulosa after 60 days of stress on plot 1D in 1975. The grasses and forbs on stress plots B, C, and D, Site 1, cured a much yellower color overwinter (1975-1976) than did adjacent non-stressed vegetation.

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# APPENDIX

The method used by the Montana State University Soils Testing Laboratory for analysis of plant sulfur content is outlined below. The method is described in Stoltenberg (1969).

This procedure is a modification of one that originally appeared in: Bardsley, D.E. and J.D. Lancaster. Determination of Reserve Sulfur and Soluble Sulfates in Soils. Soil Science Society of America Proceedings 24: 265-268. July-August, 1960.

1. Grind dry plant material in a Wiley mill, with 20-mesh screen.

2. Mix 0.250 g plant + 1 g ashing powder in 50 ml beaker.

3. Add 0.5 g ashing powder as topdressing to minimize loss of volatiles.

   a. Ashing powder: 27 g $Na_2CO_3$
      3 g $NaHCO_3$
      1.8 g $NaNO_3$
      0.06 g $KMnO_4$

   b. These are ground in power mortar and pestle until no greater color intensity is observed from $KMnO_4$ (about 5 minutes).

4. Beakers are set in a muffle furnace for 1 hour at 260°C, then 2 hours at 480°C.

5. Ash is treated with 25 ml acid solution.

   Acid solution: a. 114.2 ml conc HCl
      125.6 ml conc $CH_3COOH$
      8.3 g $NaH_2PO_4 \cdot H_2O$

   b. Make to 2 liters with distilled water.

6. Ash and acid are shaken for 30 minutes.

7. 5 drops of $H_2O_2$ are added to each flask for clearing.

8. Mixture is filtered through Whatman #40 double acid washed filter paper.

9. 10 ml filtrate is pipetted into 125 ml Erlenmeyer flask, and 1 ml acid seed solution is added.

Acid seed solution: 0.5434 g $K_2SO_4$ in 1 liter 6N HCl until 20 ppm S.

10. 0.5 g $BaCl_2$ is added to filtrate.

11. Flasks rest for 1 minute, then swirled until $BaCl_2$ crystals dissolve.

12. Solution is read in Bausch & Lomb colorimeter within 2-8 min. at 420 nm.

## SECTION 13

# EFFECTS OF LOW-LEVEL $SO_2$ EXPOSURE ON SULFUR ACCUMULATION AND VARIOUS PLANT LIFE RESPONSES OF SOME MAJOR GRASSLAND SPECIES UNDER FIELD CONDITIONS

by

C. C. Gordon, P. M. Rice, and P. C. Tourangeau

## INTRODUCTION

During the 1975 growing season, $SO_2$ fumigations of a cool season-short grass ecosystem were initiated on ZAPS I (Zonal Air Pollution System) from May 28 to September 28. Because of the visible changes across the four plots (0, 2, 5, and 10 pphm ) on ZAPS I in October of 1975 which were not accompanied by any significantly measurable changes in biomass or species diversity (Dodd et al., 1976), a substantial portion of the previous efforts and studies on the ponderosa pine-skunkbush sites were redirected to the ZAPS studies during 1976. The original 1975 hypothesis for the ZAPS fumigation studies was that most plant species fumigated continuously with MEDIUM and HIGH $SO_2$ concentrations on these plots during the growing season would be severely damaged or killed outright. This hypothesis was based on the $SO_2$-damaged forests studied by Linzon (1971, 1973a) and Dreisinger (1965, 1970) in the vicinity of Sudbury and Biersdorf, Ontario, Canada, where the highest $SO_2$ annual concentration was .044 ppm (4.4 pphm). The investigators also considered studies done in Anaconda, Montana, where a large stationary source of $SO_2$ emissions is located and where severe air pollution damage to vegetation occurs each year; those sites which record the highest $SO_2$ readings average only .02 ppm or less annually. Thus the hypothesis that the cool season-short grass ecosystem on the ZAPS plots would be severely damaged or killed outright on the higher $SO_2$ delivery plots was based on air pollution studies in these other two polluted areas.

Thus far the 1975 hypothesis has not proved to be valid, so a new hypothesis was formulated before the 1976 growing season began to test in the 1976 field studies on the ZAPS sites. This new hypothesis was that the plant species of cool season-short grass ecosystems which are fumigated continuously with $SO_2$ during the growing season will manifest early senescence, loss of viable seed production, and excessive quantities of sulfur throughout all plant parts (roots, stems and leaves).

To test this hypothesis, the investigators established several study objectives which encompass the following: (1) Bimonthly collections of five to six species of plant foliage during the growing season; (2) collection of roots of two grass species (one rhizomatous and one bunchgrass species) at mid and late growing season periods; (3) analysis of all plant parts for sulfur concentrations; (4) data gathering on leaf emergence, growth, and death of western wheatgrass (*Agropyron smithii*) on all plots at both the ZAPS I and II sites during the growing season to ascertain any difference in phenology by $SO_2$ concentrations, and (5) collection of mature seeds from various species of grasses, fumigated with the different $SO_2$ concentrations, at the end of the growing season for seed germination tests under laboratory conditions.

## MATERIALS AND METHODS

### 1975 VEGETATION COLLECTIONS

ZAPS I first was sampled on June 28. On September 28, both ZAPS I and the area where ZAPS II was to be built were sampled. Each of the four treatment plots on ZAPS I was subdivided into six subplots. A composite grab sample of each species from each treatment was made by collecting material from each subplot. The June sample was composed predominantly of live vegetation while the September sample consisted of cured vegetation, except the *Gutierrezia sarothrae* and *Artemisia tridentata* samples which included both green and dead tissue. Samples included flowering parts as well as vegetative portions. Chemical analysis was performed for both sulfur and fluoride.

### 1976 VEGETATION COLLECTIONS

#### Species Studied for Sulfur Accumulation

In April, ZAPS I was surveyed visually for sampling purposes to determine species present on the plots and their relative abundance and frequency. This survey was used to select the species to be studied. Those finally chosen were:

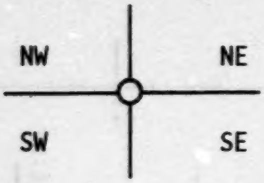
| SCIENTIFIC NAME | COMMON NAME |
|---|---|
| *Agropyron smithii* | Western Wheatgrass |
| *Koeleria cristata* | Prairie Junegrass |
| *Aristida longiseta* | Red Threeawn |
| *Poa sandbergii* (secunda) | Sandberg's Bluegrass |
| *Achillea millefolium* | Yarrow |
| *Artemisia frigida* | Fringed Sage |
| *Tragopogon dubius* | Yellow Salsify |
| *Stipa viridula* | Green Needlegrass |

*T. dubius* was collected in early July. ZAPS II was not surveyed so intensively.

*A. longiseta* was less frequently found so *S. viridula* was collected instead.

#### Collection Methods at ZAPS Sites

Pipes delivering the sulfur dioxide and ambient air mixture to the individual treatment plots were laid out in a pattern used to form the basis for a sampling grid (Figure 13.1). The pipe junctions were used as 25 sampling points. Each junction was divided further into four quadrants:



Two digit random numbers were drawn (00 to 99) and divided by four. The whole number obtained gave the junction number and the remainder gave the quadrant (1 = NE, 2 = SE, 3 = SW, 0 = NW) to be sampled.

random number ÷ 4 = junction + quadrant

37 ÷ 4 = 9 + 1 (9-NE)

88 ÷ 4 = 22 + 0 (22-NW)

etc.

Quadrants falling north of junctions 1, 11, and 21 were considered outside the treatment plot, and their respective south quadrants were used for sampling (i.e., 1-NW, 11-NW, and 21-NW became 1-SW, 11-SW, and 21-SW; 1-NE, 11-NE, and 21-NE became 1-SE, 11-SE, and 21-SE, respectively). Quadrants falling south of junctions 5, 6, 15, 16, and 25, were similarly rotated to their respective north quadrants to place them in the treatment plot.

This procedure divided each treatment plot into 86 subplots for vegetation sampling. A central sampling point was established in each "junction-quadrant" (subplot) by running a line at a 45° angle from each junction into the appropriate quadrant. The line was of a fixed length randomly determined for each collection period within the restraints of each research team's utilization of the treatment plots. Appropriate species closest to this point were then collected. No samples were collected within one meter of the pipe.

Ten subplots were chosen for each treatment plot during each collection period. Material was collected from three separate plants for most species in each subplot, resulting in one combined sample of each species derived from 30 individual plants. *Aristida longiseta* was collected from two plants per subplot, giving a sample derived from 20 individual plants. *Artemisia frigida* was less frequently found, and the sample was derived from 6 to 29 individual plants depending on the sample period.

Samples were separated by subplots during early April for *Agropyron smithii* and early July and mid-September for both *A. smithii* and *K. cristata*. This provided ten samples for each type of material (below-ground parts, dead and live tops) per species on each treatment plot. Each sample was composed of material derived from a minimum of three plants in each subplot.

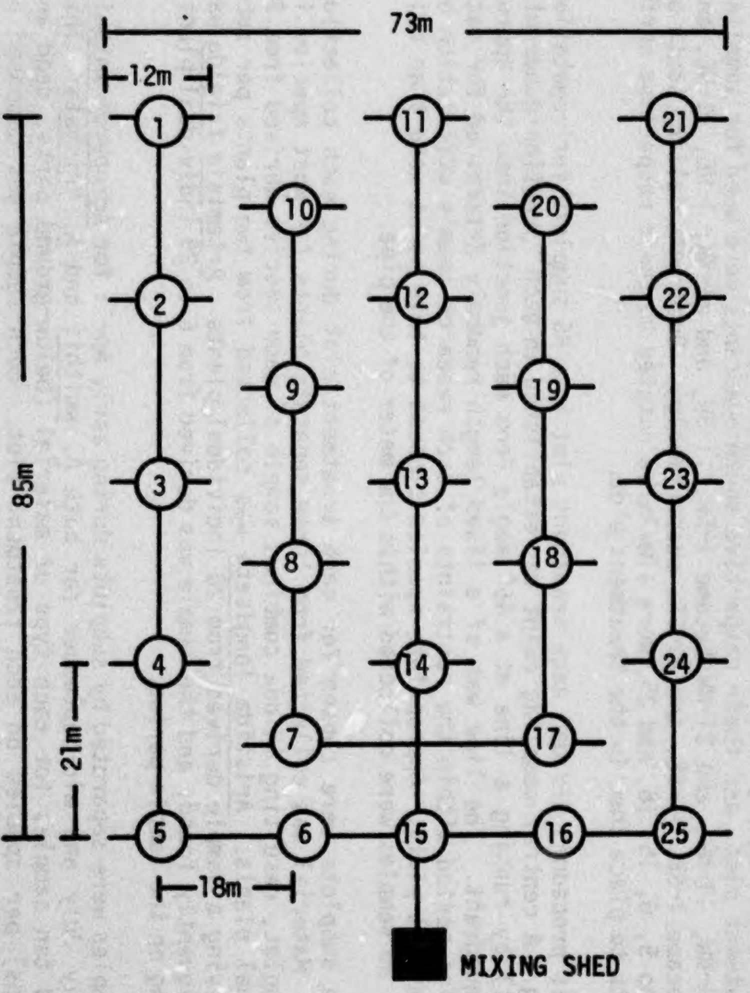

73m
12m
85m
21m
18m
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
MIXING SHED

Figure 13.1A. ZAPS plot sampling junction numbers.

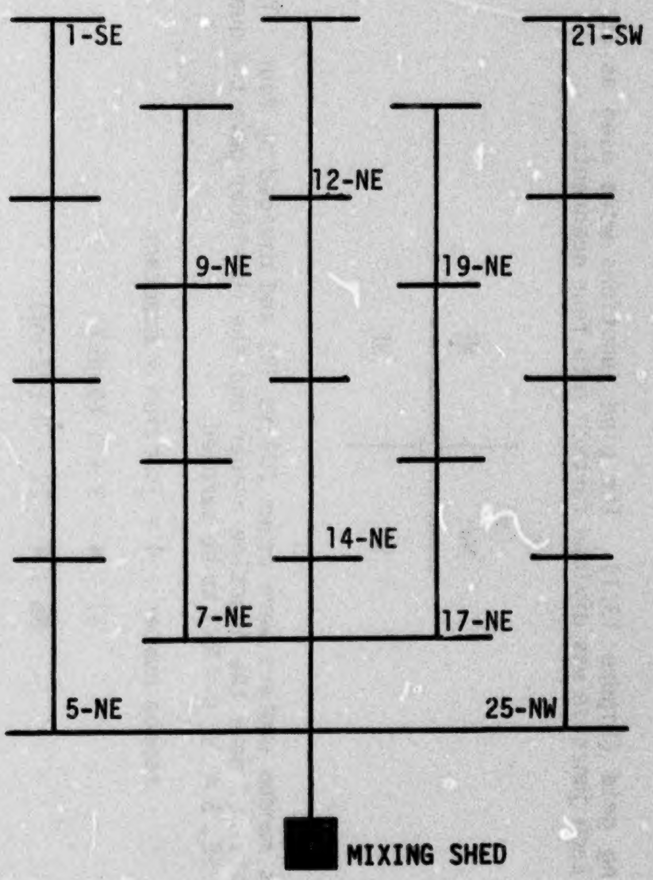

1-SE
5-NE
9-NE
7-NE
12-NE
14-NE
19-NE
17-NE
21-SW
25-NW
MIXING SHED

Figure 13.1B. *Agropyron smithii* phenology subplot locations.

Tops consisted of vegetative culms only for A. smithii, K. cristata, and S. viridula; both vegetative and reproductive culms for P. sandbergii and Aristida longiseta, and leaves only for Artemisia frigida and Achillea millefolium. T. dubius samples were split into leaves, stems, and taproots. Top material was classified as live or dead based on color (green = live, non-green = dead). Parts of partially necrotic leaf blades were placed in appropriate live or dead categories. Cocking (1973) in a field $SO_2$ fumigation study used a similar classification of live and dead.

Below-ground parts for A. smithii consisted of roots, rhizomes, and crowns to a depth of 15 cm. Weaver (1958) reported 55 percent of the root weight of A. smithii was found in the top 15 cm of soil. Jorgensen (1970) observed similar root patterns in central Montana. Below-ground parts of K. cristata consisted of roots and crowns also collected to a depth of 15 cm. Below-ground parts will be referred to as roots. Roots were washed with tap water to remove soil prior to preparation for sulfur analyses.

There were ten sample periods on ZAPS I from early April through mid-October and nine sample periods on ZAPS II from early April through mid-September. Sampling was done at approximately two-week intervals. Sulfur analysis was performed with the Leco induction furnace.

### Collection Methods at Drift Sites

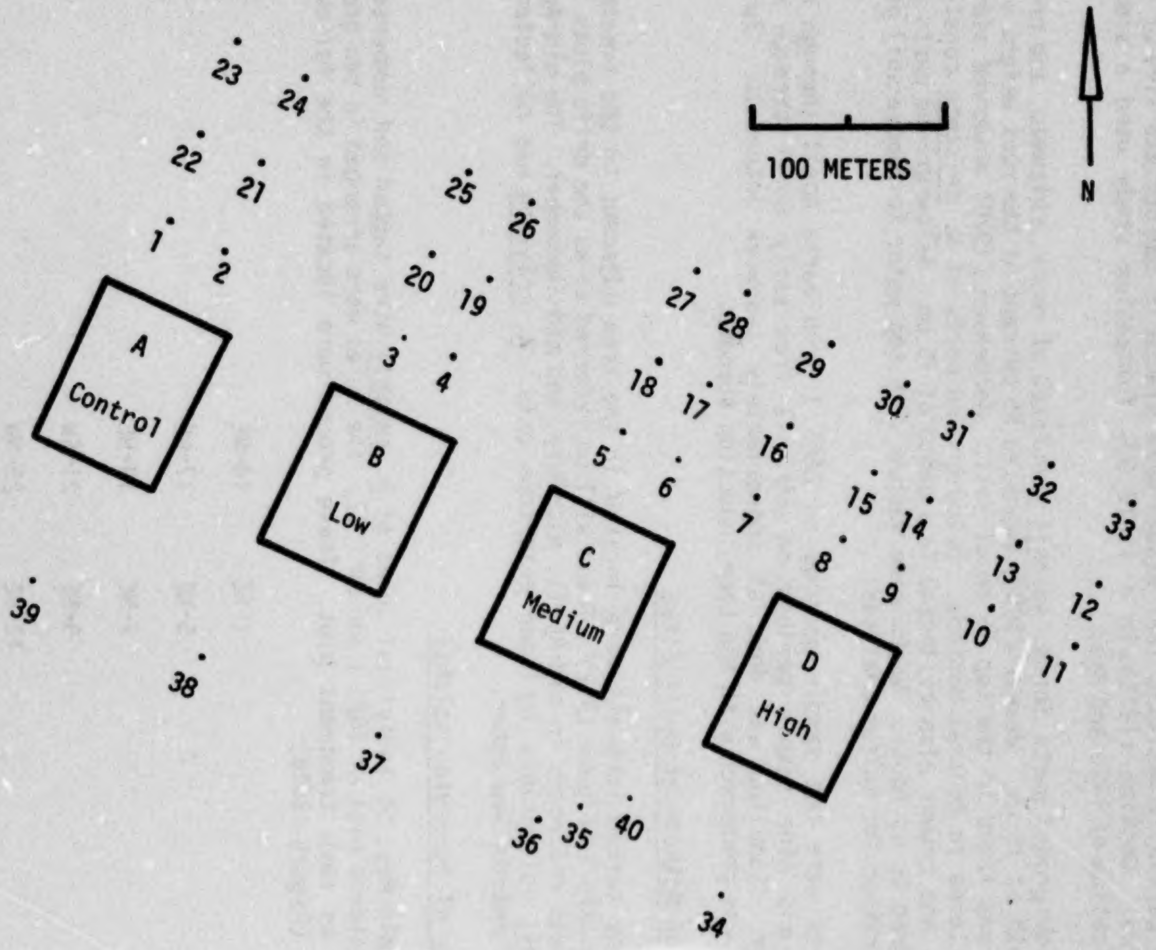
Forty marked subplots were located in the area adjacent to the treatment plots on ZAPS I (Figure 13.2). These will be referred to as the drift plots. A. smithii was collected in mid-April, mid-July, and mid-September. The mid-April A. smithii collections included dead tops only. K. cristata was collected in mid-July and mid-September.

### Phenology of Agropyron smithii

In mid-May, 50 individual culms of A. smithii were tagged and numbered on each treatment plot of ZAPS I and ZAPS II. The culms were arranged in ten groups of five on each treatment plot. These groups were located in the following subplots (Figure 13.1B):

1-SE
5-NE
7-NE
9-NE
12-NE
14-NE
17-NE
19-NE
21-SW
25-NW

The culms were marked with a numbered staking flag two inches north of the respective culms. Each culm was encircled by a twist-tie which led to the base of the flag. It is felt that neither the staking flag nor the twist-tie interfered with the growth of the culm. Jorgensen (1970) utilized a similar


100 METERS
N
A
Control
B
Low
C
Medium
D
High
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
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25
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27
28
29
30
31
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34
35
36
37
38
39
40

Figure 13.2. ZAPS I drift plot locations.

method of marking individual A. smithii culms and also concluded the effect was negligible.

The specific culms were selected by measuring five feet north from the appropriate junction along the pipe and then five feet east (west on subplots 21 and 25). The A. smithii culm closest to this point was #3 for that subplot. The culm closest to a point six inches west of this was #2, and 12 inches west was #1. Similarly #4 and #5 were selected on the east side of #3. This was repeated ten times on each treatment plot.

Data collected for the first period (mid-May) were the total number of emergent leaves, excluding totally necrotic basal leaves. The twist-tie was placed on the adaxial side of the highest totally necrotic blade if any were present. Subsequent inspections involved recording the emergent leaf-blade number and the number of total necrotic leaf-blades on each culm at approximately two-week intervals. Sampling was discontinued in mid-September. There were eight observation periods on ZAPS I and seven observations on ZAPS II.

### Seed Collection and Viability Studies

Seeds of A. smithii, K. cristata, P. sandbergii, T. dubius, and S. viridula were collected from each treatment plot on ZAPS I and II. Seeds of A. smithii and T. dubius were also collected from a similar vegetation community approximately 1.6 km southwest of the ZAPS sites.

Each seed collection was pre-treated according to the technique utilized by Eddleman (1977) for each species to enhance germination. Tetrazolium staining was conducted on the seed collections to provide seed viability information according to the rules of the Association of Official Seed Analysts (1970).

Germination tests were conducted to determine the percent germination per gram weight of seeds collected. Weights of 100 seeds were calculated to adjust the gram weights in the event that seed size is reduced as a result of the $SO_2$ treatments. Two millimeters of hypocotyl growth were considered successful germination. Seed germination was checked on a daily basis for 30 days. From this information, an "energy peak" and percent germination was determined for each seed sample. Analysis of variance and Duncan's (1955) multiple range test are being conducted on the results.

Seed germination and viability investigations are not far enough along at this time to present results or discussion on this portion of the 1976 ZAPS studies.

## RESULTS

### 1975 SULFUR AND FLUORIDE ACCUMULATION AT ZAPS I

On Table 13.1 are the sulfur and fluoride concentrations in foliage of ten species of grasses, forbs, and shrubs collected from the ZAPS I plots during 1975. Only two grass species (i.e., A. smithii and B. japonicus) were collected during both the June and September collection periods. Sulfur levels in these two grass species increased four times in A. smithii and three times in B.

TABLE 13.1. ZAPS PPM SULFUR (S) AND PPM FLUORIDE (F) LEVELS FOR 1975.

| ZAPS I | 28 June 1975 (31 Days Fumigation) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | A (CONTROL) | | B (LOW) | | C (MEDIUM) | | D (HIGH) | |
| | S | F | S | $F^-$ | S | $F^-$ | S | $F^-$ |
| *Agropyron smithii* | 1200 | 3.6 | 1500 | 2.6 | 1600 | 2.7 | 2000 | 1.8 |
| *Koeleria cristata* | 800 | 4.6 | 1050 | 3.9 | 1200 | 2.0 | 800 | 1.3 |
| *Achillea millefolium* | 600 | 3.2 | 900 | 3.3 | 1200 | 2.3 | 2200 | 3.5 |
| *Bromus japonicus* | 1175 | 2.7 | 1200 | 4.2 | 2200 | 2.9 | 2550 | 2.8 |
| *Poa pratensis* | 600 | 1.4 | 1100 | 2.8 | 1200 | 2.7 | 1400 | 1.9 |

| ZAPS I | 28 September 1975 (123 Days Fumigation) | | | | | | | | ZAPS II Pre-Treatment | |
|---|---|---|---|---|---|---|---|---|---|---|
| | A (CONTROL) | | B (LOW) | | C (MEDIUM) | | D (HIGH) | | | |
| | S | F | S | $F^-$ | S | $F^-$ | S | $F^-$ | S | F |
| *Agropyron smithii* | 850 | 1.0 | 1800 | 1.4 | 3400 | 0.8 | 5250 | 0.8 | 900 | 0.8 |
| *Bromus japonicus* | 700 | 2.5 | 1600 | 1.0 | 2400 | 0.4 | 3400 | 1.1 | 600 | 1.3 |
| *Aristida longiseta* | 700 | 0.8 | 1700 | 1.6 | 1300 | 1.7 | 3300 | 0.1 | 800 | 1.2 |
| *Artemisia tridentata* | 1300 | 1.6 | 1200 | 2.4 | 1400 | 1.8 | 2750 | 1.2 | 1200 | 2.1 |
| *Bromus tectorum* | 400 | 0.5 | 1500 | 0.5 | 2100 | 1.7 | 4150 | 0.8 | 400 | 1.5 |
| *Stipa comata* | 900 | 0.9 | 1800 | 1.0 | 2800 | 1.4 | 2800 | 0.8 | 800 | 0.1 |
| *Gutierrezia sarothrae* | 1700 | 2.4 | 1500 | 1.9 | 2950 | 1.0 | 5300 | 1.3 | 2200 | 1.8 |

japonicus, while the fluoride levels decreased during this 90-day period. Sulfur levels in foliage of all nine species collected during the latter part of September at the four fumigation concentrations demonstrated that sulfur accumulation correlated with fumigation concentration and duration of exposure.

Because the vegetation species on the ZAPS I plot survived remarkably well during the 1975 growing season after continuous fumigation with $SO_2$, a large portion of the 1976 studies was redirected from the ponderosa pine-skunkbush sites to the ZAPS I and II sites.

## 1976 SULFUR ACCUMULATION AT ZAPS I AND II

Data analysis of sulfur accumulation in above-ground vegetation from the ZAPS sites does not include the ninth (mid-September) and tenth (mid-October) 1976 collection periods. However, the analysis covers the major portion of the growing season. This is confirmed by the 1976 phenology studies on A. smithii and the 1975 phenology studies conducted by personnel of Colorado State University (Dodd et al., 1976) for P. sandbergii, K. cristata, Aristida longiseta, A. smithii, T. dubius, and Achillea millefolium. Dormancy is not induced in Artemisia frigida until later in the season, as is typical of the sages, because this species is able to extract water from greater depths. In the presence of adequate fall rains, many prairie species resume growth. Sulfur levels in live and dead tops for the six species collected throughout the study period are presented in Appendix A.

The changes in sulfur levels through time were analyzed by fitting a linear regression to the data by least squares. An initial peak in the sulfur concept of live tops from the first collection period was observed. This spring green-up peak was noted in all species. To allow preliminary use of a simple linear model, this initial collection was excluded from the calculation of subsequent live regression lines. The coefficients for the regression lines are presented in Table 13.2. Of the 80 regression coefficients examined, 38 were found to be significant at $p \leq .10$ (Table 13.3).

The number of significant regressions increased with higher levels of fumigation. Although only two of the regressions on the A plots were significant, 18 of the 20 regressions showed a positive slope ($p < .001$), suggesting a trend of increasing sulfur levels in vegetation on the control plots from the beginning to the end of the growing season. There was a substantial increase in the average value of the Coefficient of Determination ($R^2$) from the A plots (.349) to the B plots (.635). The increase from the B's (.635) to the C's (.683) and subsequently from the C's to the D's (.743) was less. This degeneration of the increase in the goodness of fit with higher fumigation levels was due to the fact that many species reached a marked plateau of sulfur accumulation in late June through July. This phenomenon will be examined when individual species are considered.

The regression lines for live tops for six species are presented in Figures 13.3 through 13.8 (regression was not computed for P. sandbergii because it was collected during only three sample periods). The trends in sulfur accumulation can be quickly summarized from these graphs. Sulfur levels in live vegetation increased with exposure time. The rate of increase rose

TABLE 13.2. REGRESSION COEFFICIENTS FOR SULFUR LEVELS THROUGH TIME.

Agropyron smithii

| | Live | | | | | Dead | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Plot | $b_o$ | $b_1$ | $F_s$ | $R^2$ | n | $b_o$ | $b_1$ | $F_s$ | $R^2$ | n |
| Z1A | 288 | 3.27 | 3.943 | .441 | 7 | 17 | 3.90 | 3.891 | .565 | 5 |
| Z2A | 924 | -0.13 | 0.011 | .002 | 7 | -15 | 3.69 | 141.767‡ | .986 | 4 |
| Z1B | 250 | 6.00 | 44.500‡ | .889 | 7 | -433 | 8.88 | 13.503† | .818 | 5 |
| Z2B | 992 | 2.06 | 1.501 | .231 | 7 | -287 | 8.97 | 16.006* | .889 | 4 |
| Z1C | 102 | 9.96 | 13.633† | .731 | 7 | -917 | 15.47 | 11.231† | .789 | 5 |
| Z2C | 120 | 10.20 | 13.839† | .735 | 7 | -1638 | 20.76 | 67.929† | .971 | 4 |
| Z1D | -792 | 18.53 | 775.724‡ | .994 | 7 | -1479 | 22.50 | 5.731* | .656 | 5 |
| Z2D | 1410 | 11.19 | 1.813 | .266 | 7 | -2346 | 30.27 | 7.909 | .798 | 4 |

Koeleria cristata

| | Live | | | | | Dead | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Plot | $b_o$ | $b_1$ | $F_s$ | $R^2$ | n | $b_o$ | $b_1$ | $F_s$ | $R^2$ | n |
| Z1A | 479 | 1.37 | 1.242 | .199 | 7 | 530 | 0.31 | 0.109 | .035 | 5 |
| Z2A | 660 | 0.25 | 0.030 | .006 | 7 | 382 | 1.10 | 3.315 | .624 | 4 |
| Z1B | -218 | 8.38 | 18.022‡ | .783 | 7 | -288 | 7.73 | 136.236‡ | .978 | 5 |
| Z2B | 307 | 4.38 | 3.927 | .440 | 7 | 12 | 5.56 | 30.128† | .938 | 4 |
| Z1C | 5 | 10.86 | 22.647‡ | .819 | 7 | 27 | 8.17 | 8.187* | .732 | 5 |
| Z2C | 219 | 10.08 | 9.562† | .657 | 7 | -509 | 11.19 | 19.730† | .908 | 4 |
| Z1D | -19 | 16.58 | 5.394* | .519 | 7 | -8 | 11.32 | 20.310* | .871 | 5 |
| Z2D | -1110 | 22.12 | 7.257† | .592 | 7 | -1246 | 19.66 | 11.659* | .854 | 4 |

* = $.05 \leq p \leq .10$

† = $.01 \leq p \leq .05$

‡ = $p \leq .01$

(continued)

TABLE 13.2. (Continued)

| | | | | *Achillea millefolium* | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Live | | | | | | Dead | | | | |
| Plot | $b_o$ | $b_1$ | $F_s$ | $R^2$ | n | $b_o$ | $b_1$ | $F_s$ | $R^2$ | n |
| Z1A | 339 | 1.78 | 1.496 | .230 | 7 | 634 | 0.13 | 3.138 | .007 | 5 |
| Z2A | 907 | -0.75 | 0.351 | .066 | 7 | 494 | 0.75 | 2.194 | .422 | 5 |
| Z1B | 542 | 2.26 | 1.208 | .195 | 7 | 456 | 3.65 | 1.035 | .341 | 4 |
| Z2B | 1223 | -0.10 | 0.001 | <.001 | 7 | 298 | 4.91 | 8.253* | .733 | 5 |
| Z1C | 230 | 8.14 | 16.306† | .765 | 7 | 410 | 9.28 | 4.173 | .582 | 5 |
| Z2C | 852 | 5.75 | 1.468 | .227 | 7 | -831 | 16.25 | 4.921 | .621 | 5 |
| Z1D | -1883 | 26.68 | 33.490‡ | .870 | 7 | -451 | 26.64 | 6.753* | .692 | 5 |
| Z2D | -662 | 25.37 | 12.810† | .719 | 7 | -2228 | 32.57 | 14.619† | .830 | 5 |
| | | | | *Artemisia frigida* | | | | | | |
| Live | | | | | | Dead | | | | |
| Plot | $b_o$ | $b_1$ | $F_s$ | $R^2$ | n | $b_o$ | $b_1$ | $F_s$ | $R^2$ | n |
| Z1A | 1188 | 0.91 | 0.291 | .068 | 6 | 964 | 1.41 | 102.084* | .990 | 3 |
| Z2A | 1074 | 1.19 | 0.260 | .049 | 7 | 680 | 0.89 | 0.198 | .163 | 3 |
| Z1B | 2043 | -1.70 | 0.672 | .199 | 7 | 700 | 4.45 | 10.197 | .911 | 3 |
| Z2B | 832 | 4.13 | 1.841 | .274 | 7 | 758 | 2.83 | 20.120 | .953 | 3 |
| Z1C | 1087 | 7.63 | 3.428 | .461 | 6 | 1087 | 7.63 | 12.228 | .461 | 3 |
| Z2C | -1116 | 19.41 | 58.277‡ | .936 | 6 | -745 | 16.00 | 9.125 | .901 | 3 |
| Z1D | 971 | 18.29 | 6.890* | .633 | 6 | 1860 | 9.05 | 8.253 | .892 | 3 |
| Z2D | -2 | 23.01 | 26.140‡ | .867 | 6 | -1989 | 29.78 | 4.979 | .833 | 3 |

* = $.05 \le p \le .10$

† = $.01 \le p \le .05$

‡ = $p \le .01$

(continued)

TABLE 13.2. (Continued)

*Aristida longiseta*

| | Live | | | | | Dead | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Plot | $b_o$ | $b_1$ | $F_s$ | $R^2$ | n | $b_o$ | $b_1$ | $F_s$ | $R^2$ | n |
| Z1A | -238 | 4.81 | 0.926 | .481 | 3 | 207 | 2.19 | 3.151 | .612 | 4 |
| Z1B | -654 | 7.99 | 33.624 | .971 | 3 | 455 | 2.74 | 132.994‡ | .985 | 4 |
| Z1C | 1473 | -0.21 | <.001 | <.001 | 3 | 191 | 5.82 | 19.106† | .905 | 4 |
| Z1D | 112 | 9.44 | 4.928 | .831 | 3 | -41 | 8.32 | 118.471‡ | .983 | 4 |

*Stipa viridula*

| | Live | | | | | Dead | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Plot | $b_o$ | $b_1$ | $F_s$ | $R^2$ | n | $b_o$ | $b_1$ | $F_s$ | $R^2$ | n |
| Z2A | 577 | 2.20 | 2.379 | .373 | 6 | 348 | 2.16 | 4.357 | .685 | 4 |
| Z2B | 673 | 3.21 | 0.914 | .186 | 6 | -920 | 9.87 | 75.000* | .987 | 3 |
| Z2C | 479 | 9.03 | 3.869 | .492 | 6 | -1110 | 15.75 | 66.394† | .971 | 4 |
| Z2D | 1669 | 11.33 | 0.812 | .169 | 6 | -1938 | 24.11 | 116.062 ‡ | .983 | 4 |

$b_o$ = Y-Intercept, $b_1$ = Slope, $F_s = \frac{\text{Mean Square due to Regression}}{\text{Unexplained Error}}$,

$R^2$ = Coefficient of Determination, n = number of collection periods

$H_o : \beta_1 = 0, F_{\alpha[1,n-2]}$,

$* = .05 \leq p \leq .10$

$† = .01 \leq p \leq .05$

$‡ = p \leq .01$

TABLE 13.3. SUMMATION OF NUMBER OF SIGNIFICANT REGRESSION COEFFICIENTS.

| Treatment | A | B | C | D |
|---|---|---|---|---|
| Live | 0 | 2 | 6 | 7 |
| Dead | 2 | 7 | 7 | 7 |

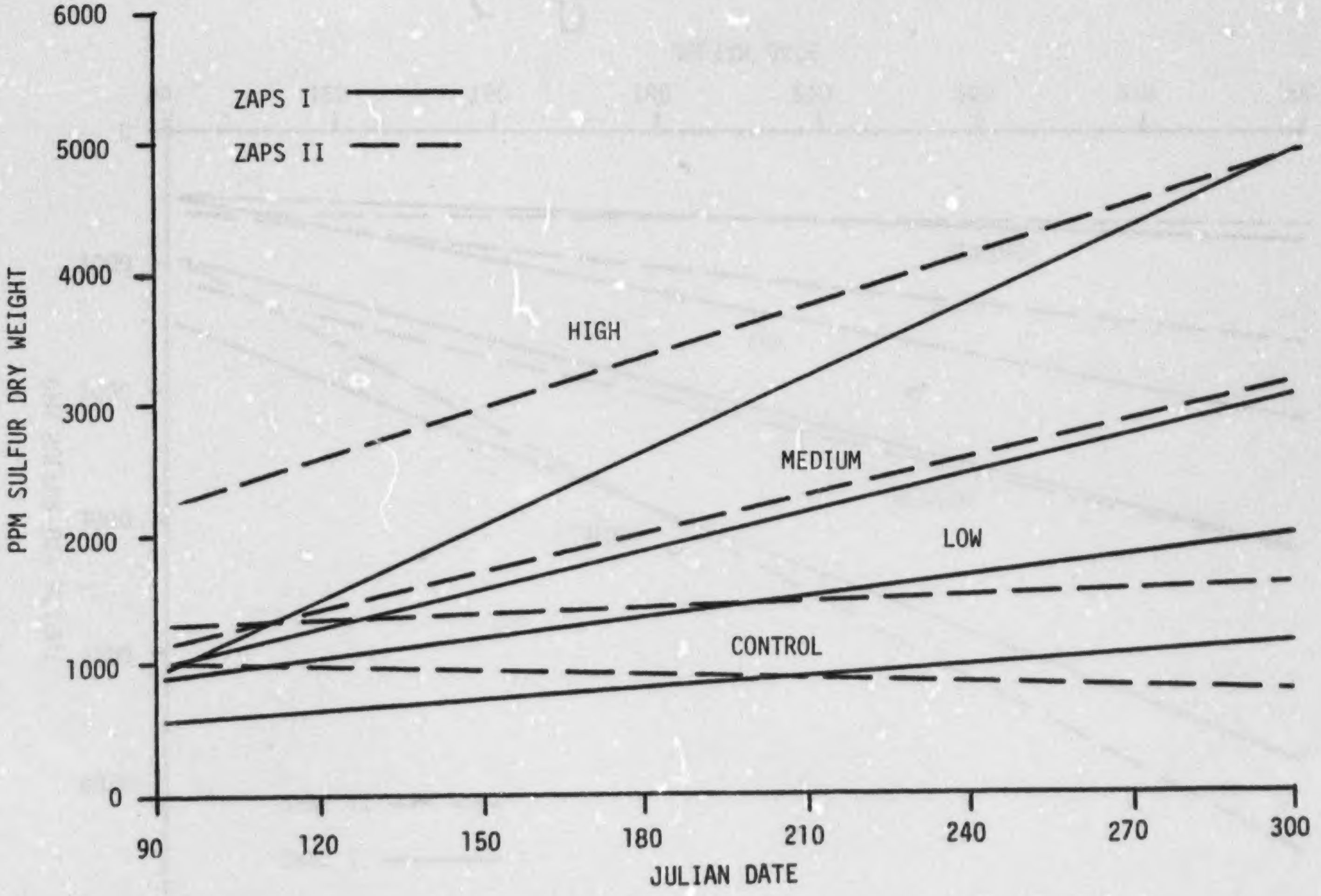

Figure 13.3. Least squares, _Agropyron_ _smithii_, live collections 2 thru 8, ZAPS I and II.

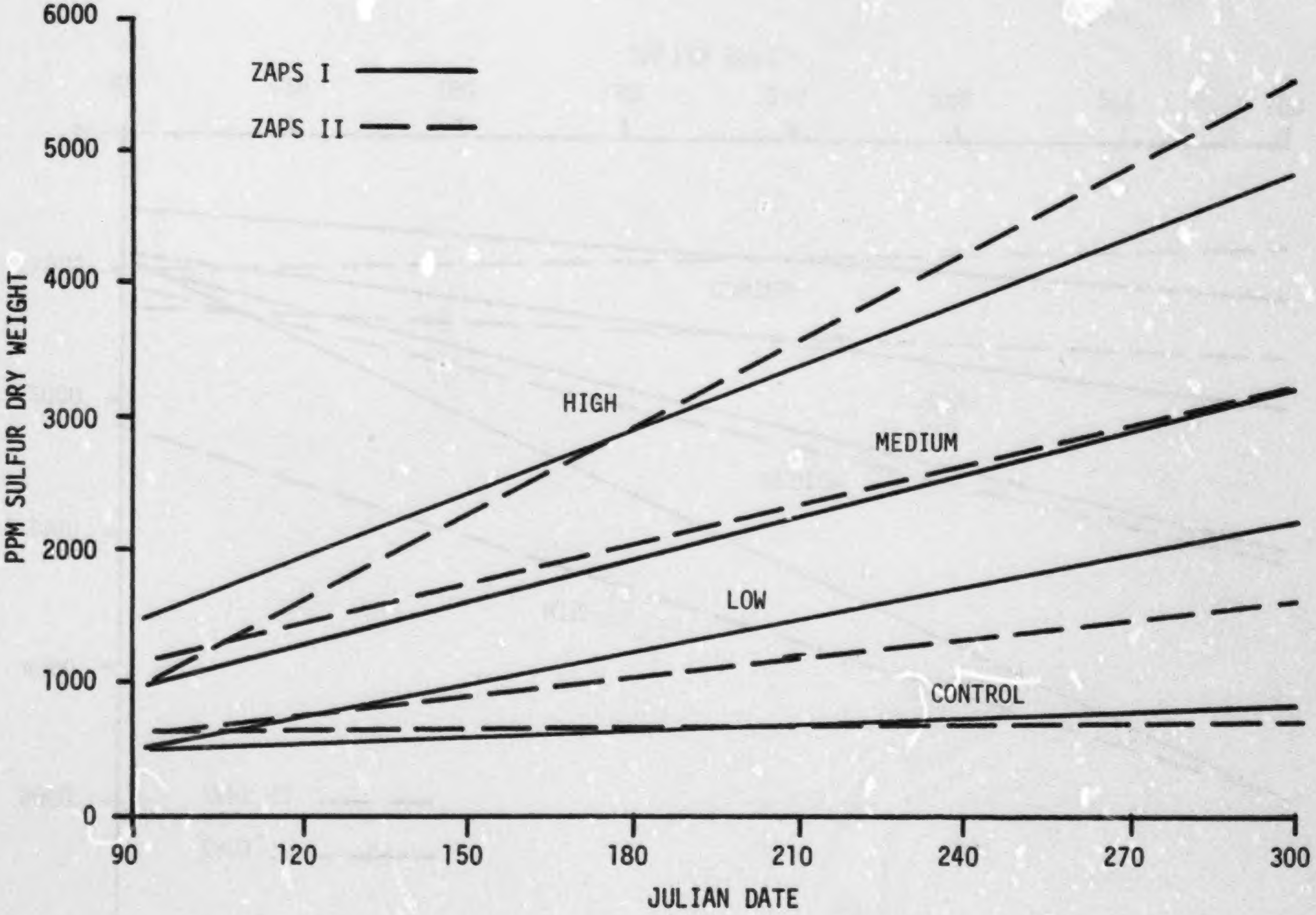

Figure 13.4. Least squares, Koeleria cristata, live collections 2 thru 8, ZAPS I and II.

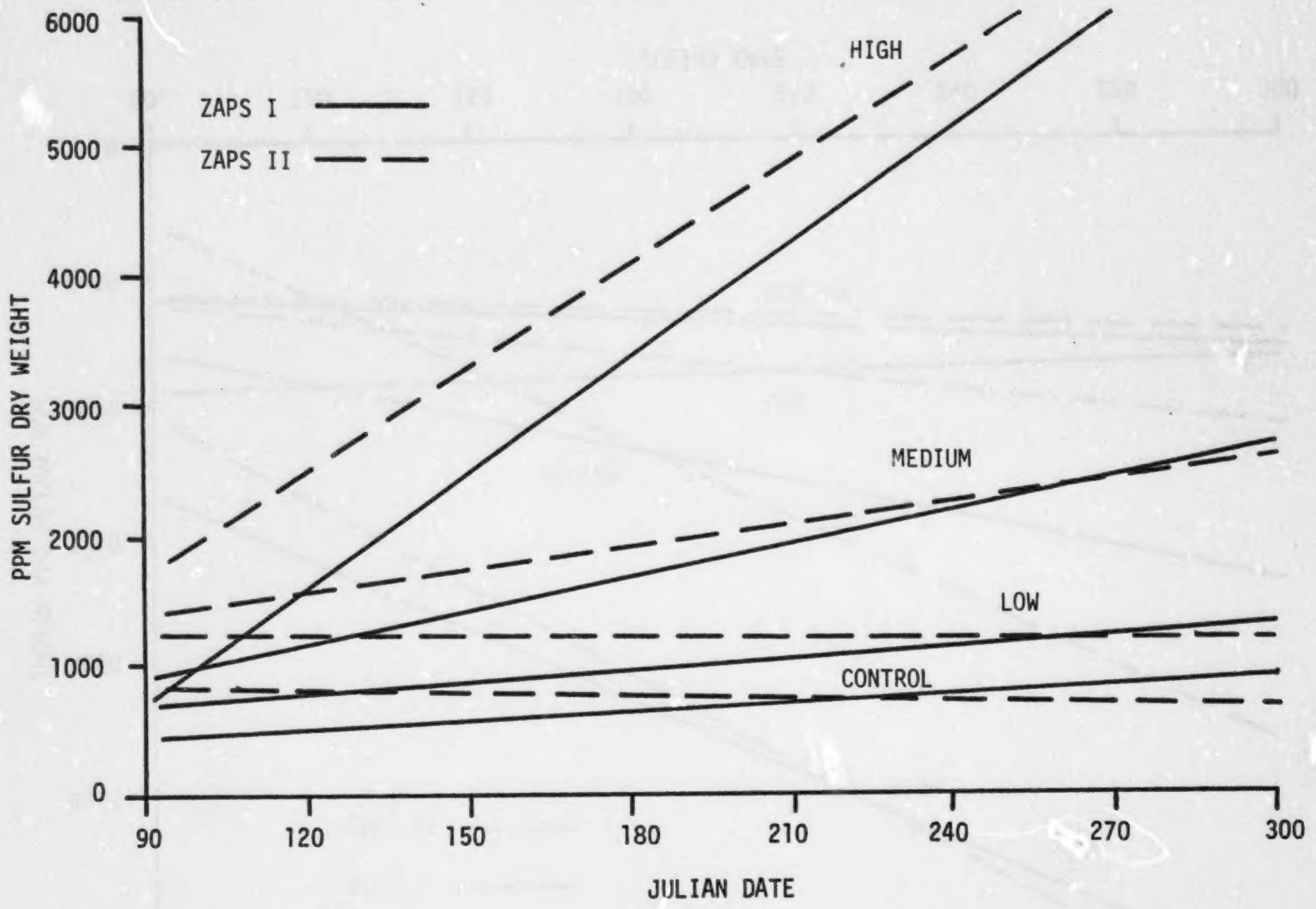

Figure 13.5. Least squares, Achillea millefolium, live collections 2 thru 8, ZAPS I and II.

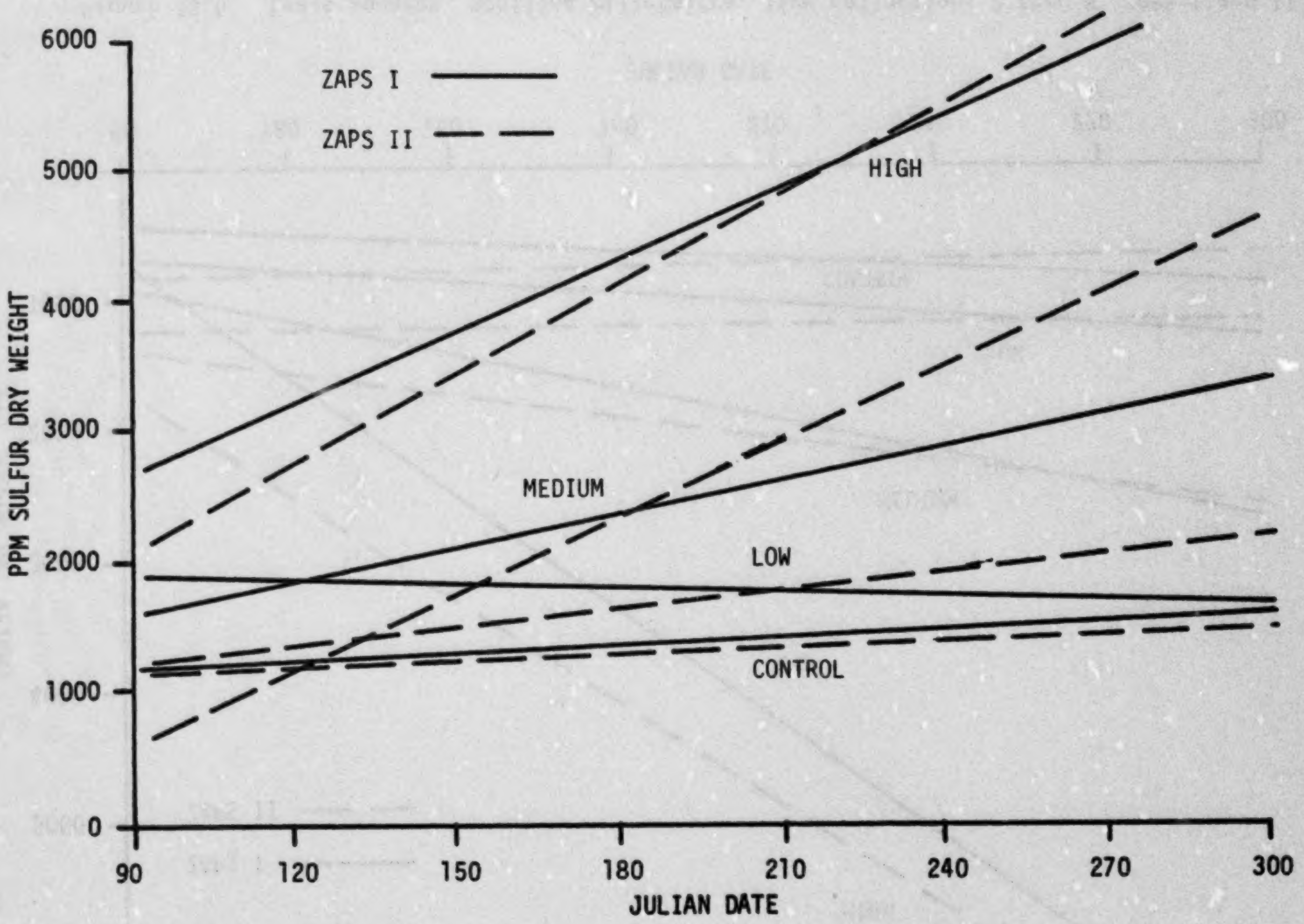

Figure 13.6. Least squares, Artemisia frigida, live collections 2 thru 8, ZAPS I and II.

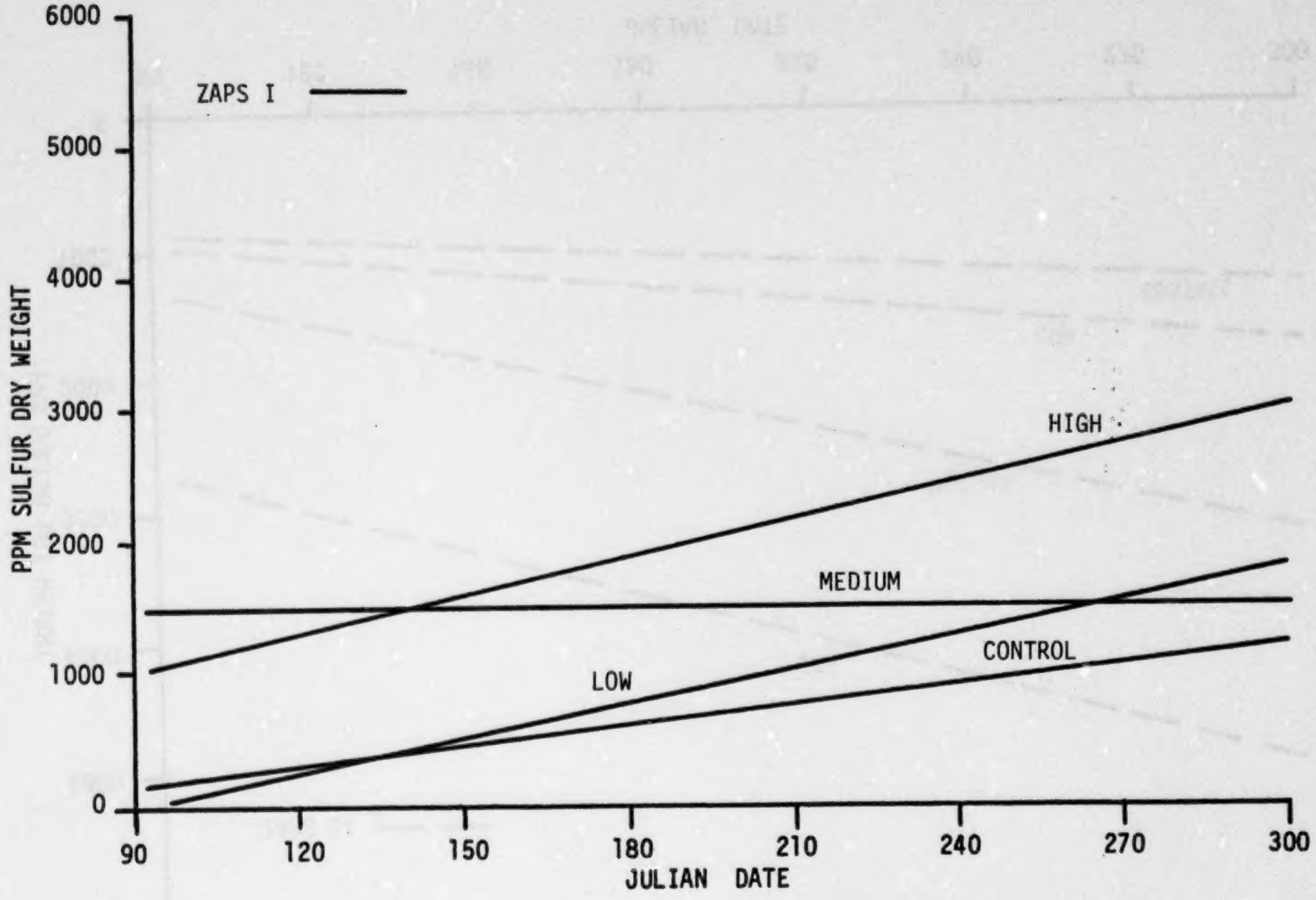

Figure 13.7. Least squares, Artistida longiseta, live collections 6 thru 8, ZAPS I.

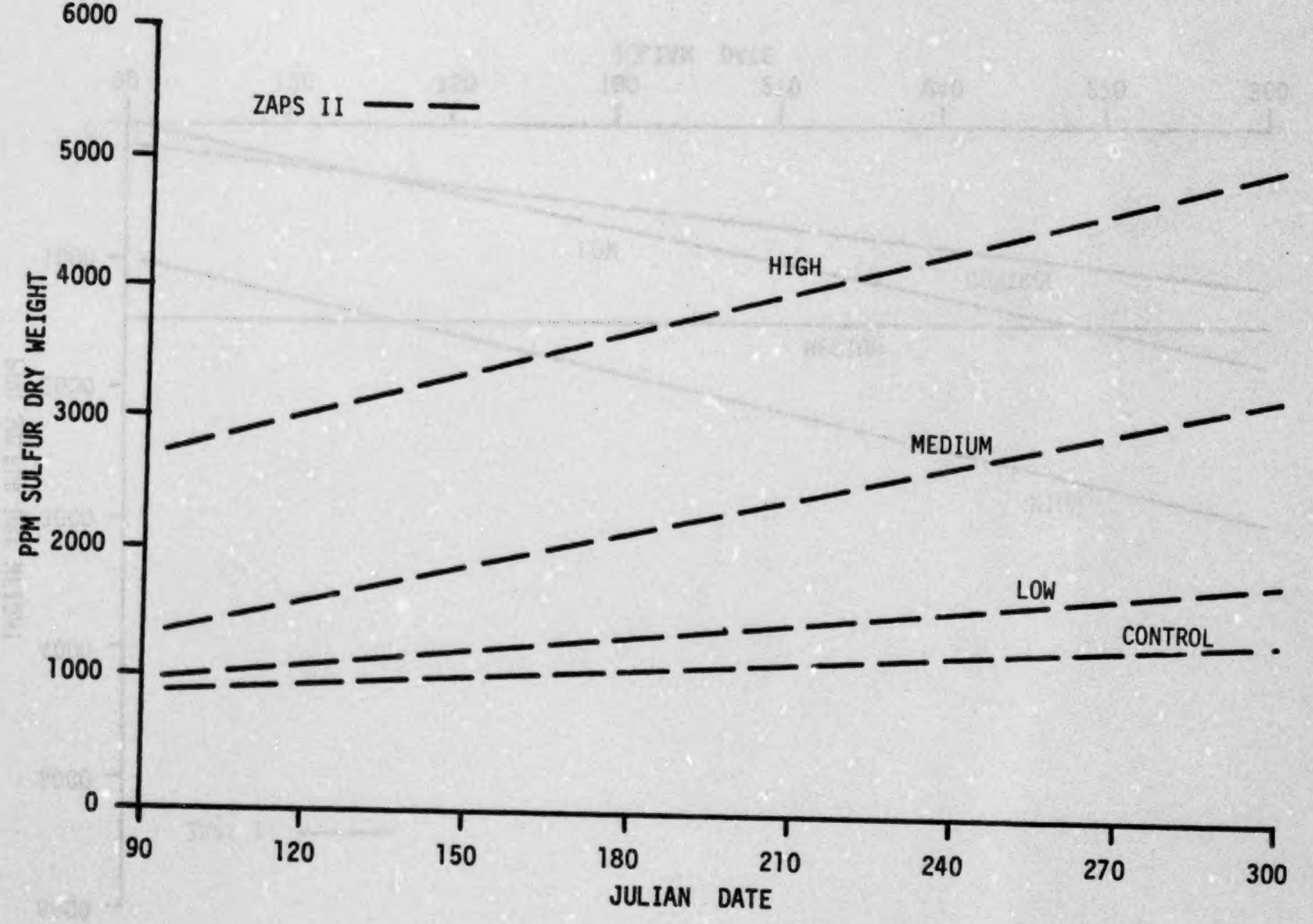

Figure 13.8. Least squares, *Stipa viridula*, live collections 3 thru 8, ZAPS II.

with increasing levels of fumigation. Rate of accumulation and total sulfur were approximately similar on ZAPS I and ZAPS II. The rate of accumulation on ZAPS II treatment plots D and C tended to be higher than on their respective ZAPS I counterparts. The rate of accumulation on ZAPS II treatment plots B and A tended to be lower than on their respective ZAPS I counterparts.

### Agropyron smithii

The observed sulfur contents and their regression lines for A. smithii, live and dead, at the various treatments are plotted in Figures 13.9 through 13.12. At treatment plot A fumigation levels the sulfur content of dead plant material remained below that of live tissue throughout most of the growing season. Sulfur levels in the dead material slowly approached the live material levels and can be expected to exceed these only in late summer as the grass comes to a full cure. As the intensity of fumigation increased, the sulfur level in dead tissue exceeded that in live tissue at an earlier date. This was indicated by both the measured sulfur content and their fitted regression. Initial sulfur levels in dead tissue were higher on ZAPS I than on ZAPS II; the sulfur content of live tissue was also higher on ZAPS I, except in vegetation from the control plots.

A plateau in the sulfur accumulation by live tissue was reached by late July, except on ZAPS I, plot D, where the rate of accumulation remained linear. This plateau occurred at a maximum sulfur level of approximately 4,000 ppm. Sulfur levels in dead tissue peaked as high as 5,675 ppm on the D fumigation plots. On May 31 A. smithii was collected from small areas immediately adjacent to the gas outlets on ZAPS II, plot D. At this time these burn areas were between 45 and 60 cm in diameter. The A. smithii collected here displayed bifacial necrotic lesions coalescing to form interveinal streaks; the streaks were ivory to light tan. Tingey et al. (1975) has described these symptoms in A. smithii treated with 150 pphm $SO_2$ in four-hour chamber fumigations. The A. smithii from these burn areas on ZAPS II, plot D contained 3,350 ppm sulfur. Similar symptoms were observed on ZAPS II, plot C but the sulfur level in the tissue was not determined specifically for A. smithii because individual species were not separated in this sample.

### Koeleria cristata

The responses of K. cristata to the treatments are graphed in Figures 13.13 through 13.16. The relationship between sulfur levels in live and dead foliage was markedly different from that which occurred for A. smithii. With K. cristata, the sulfur content of dead tissue did not exceed that of live tissue based on the regression analysis; when individual data points were considered, sulfur levels in live material were exceeded only rarely by levels in dead tissue. Sulfur levels in dead tissue can be expected to approach those in live tissue as K. cristata comes to a full cure. Sulfur levels for all tissue were similar in K. cristata and A. smithii, with a maximum of approximately 5,000 ppm and a plateau developing later in July.

A comparison of initial sulfur levels in dead K. cristata showed a residual effect similar to that of A. smithii. Sulfur levels in live K. cristata were higher at ZAPS I, plots A and B than at ZAPS II, plots A and B.

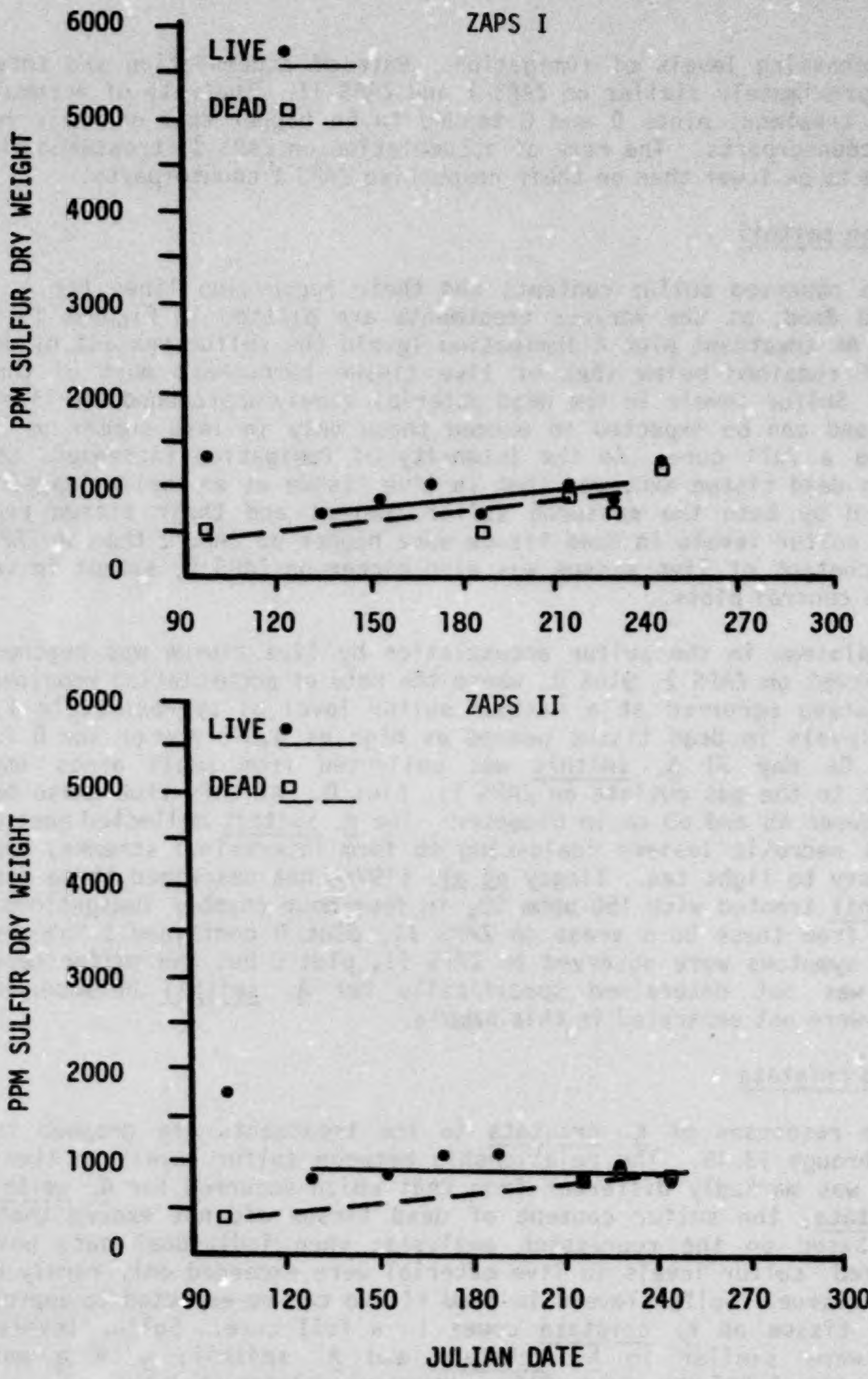

Figure 13.9. **Linear regressions of sulfur levels through time in *Agropyron smithii* at CONTROL fumigation.**

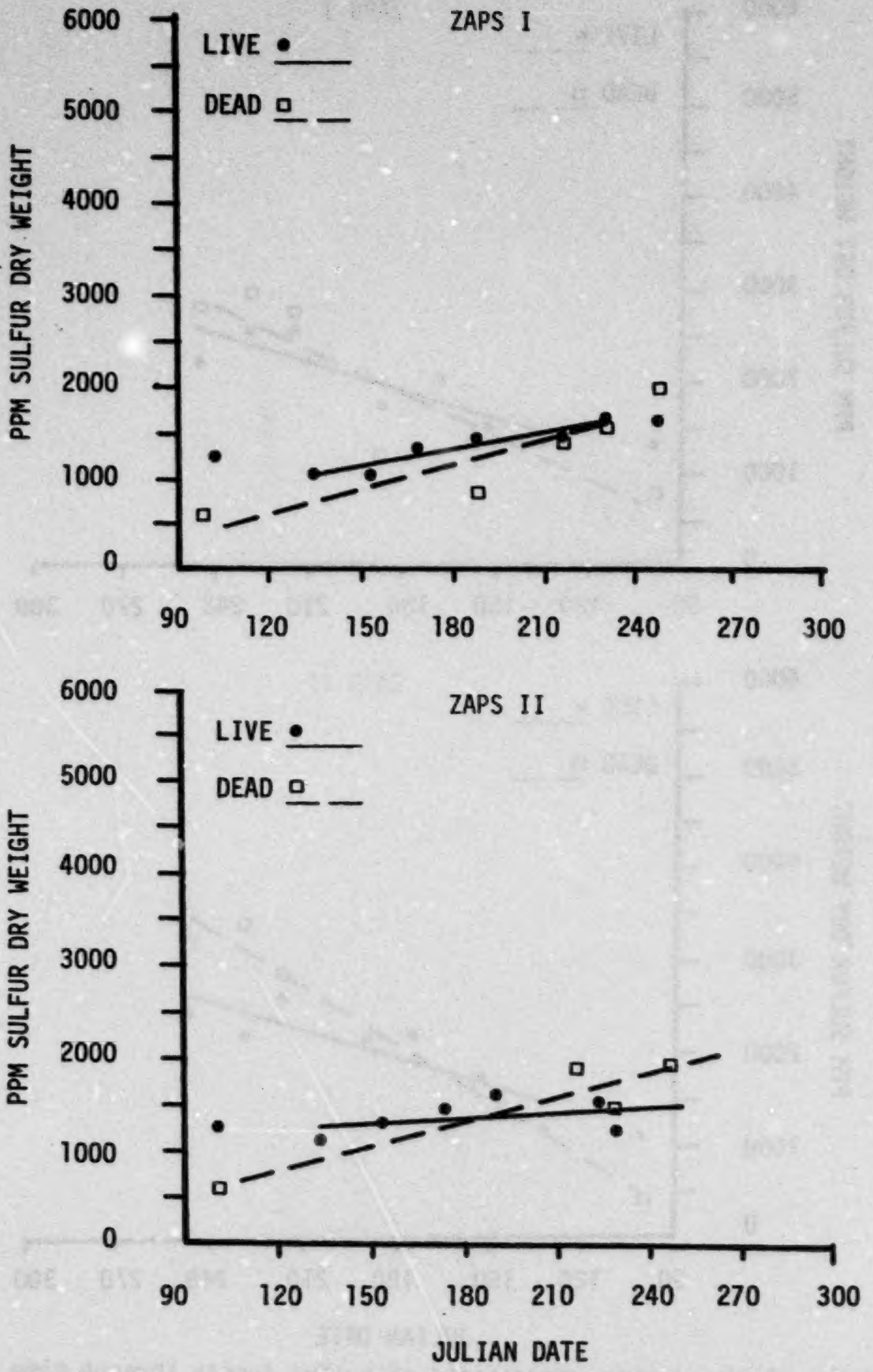

Figure 13.10. Linear regressions of sulfur levels through time in *Agropyron smithii* at LOW fumigation.

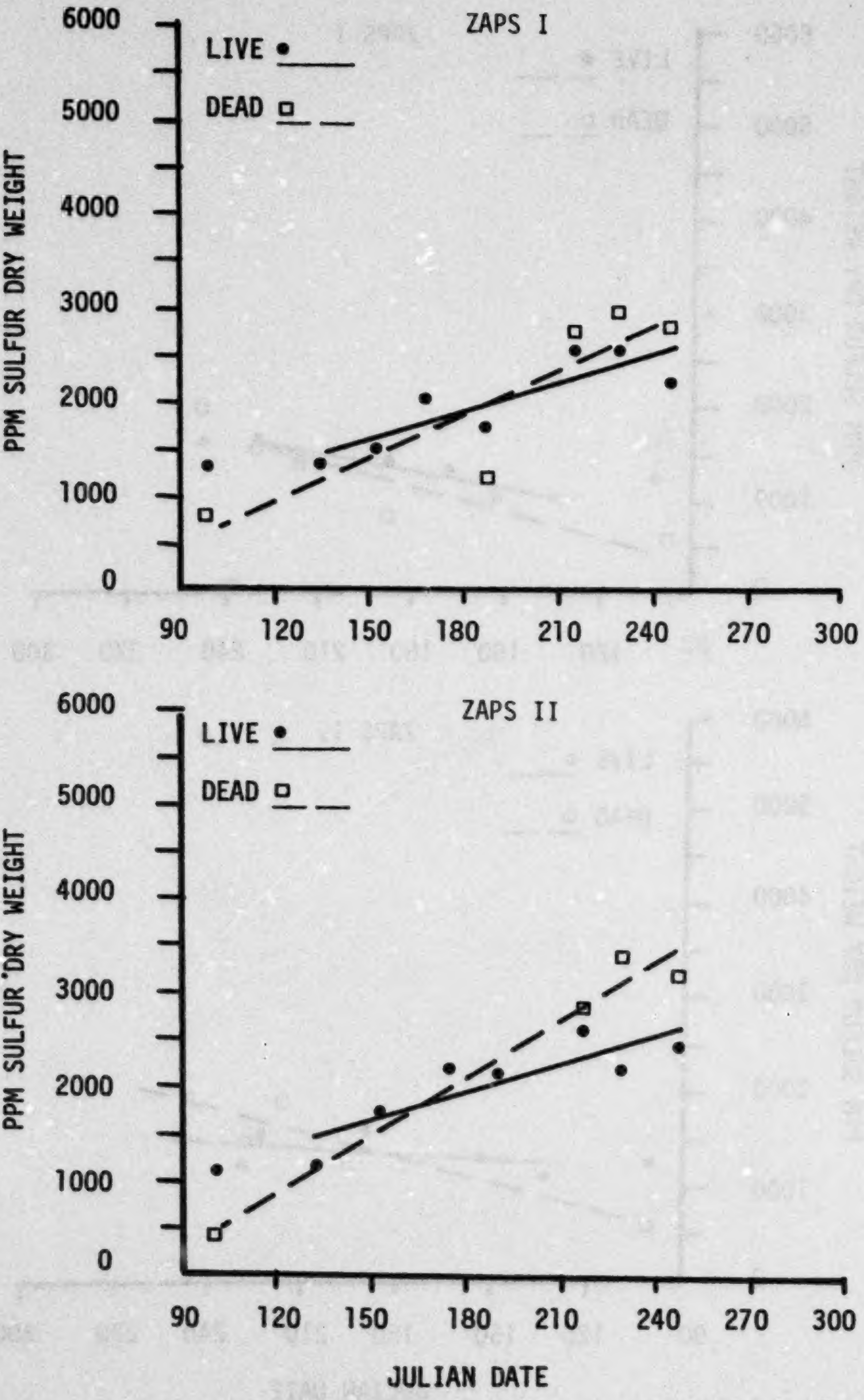

Figure 13.11. Linear regressions of sulfur levels through time in _Agropyron smithii_ at MEDIUM fumigation.

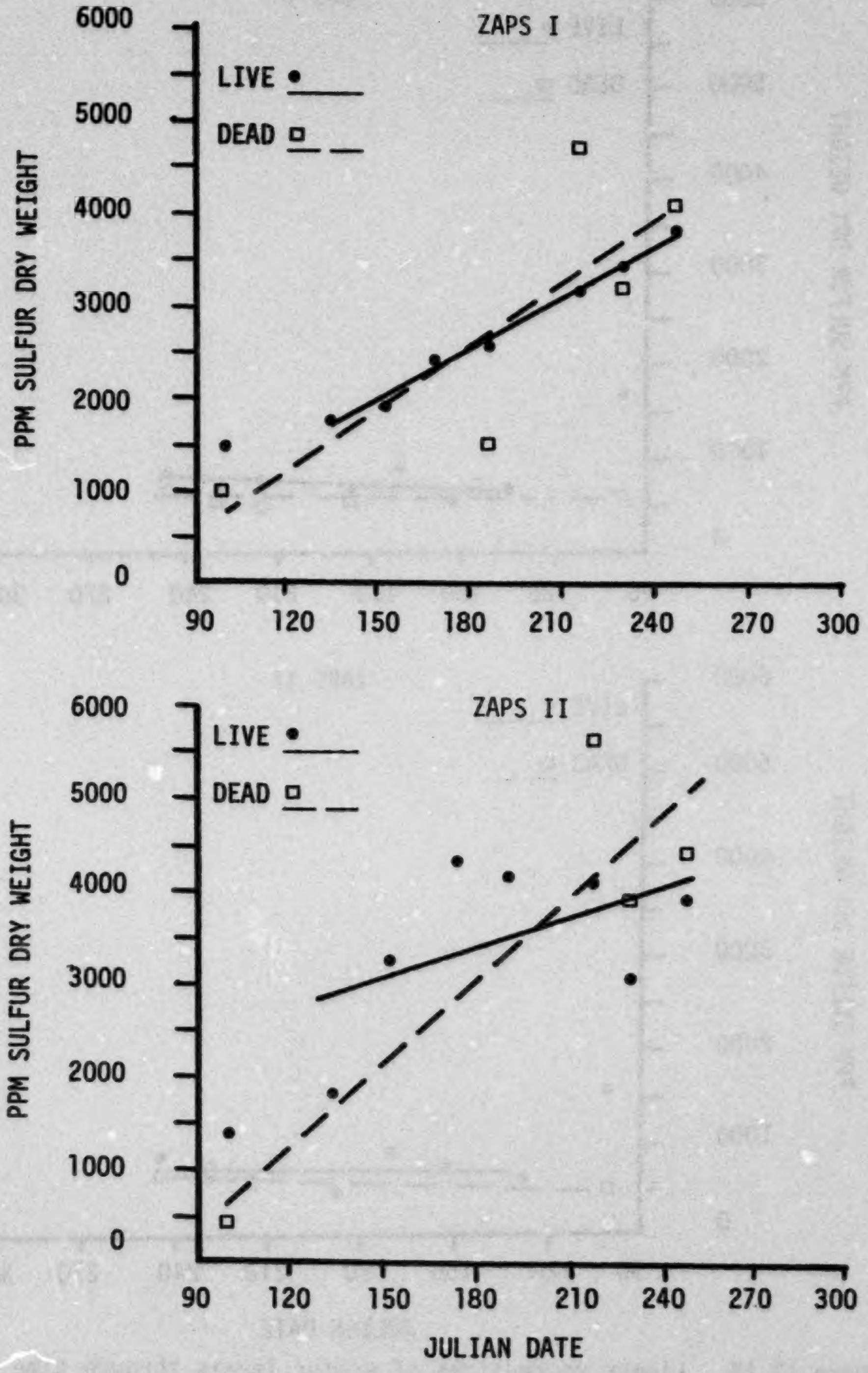

Figure 13.12. Linear regressions of sulfur levels through time in Agropyron smithii at HIGH fumigation.

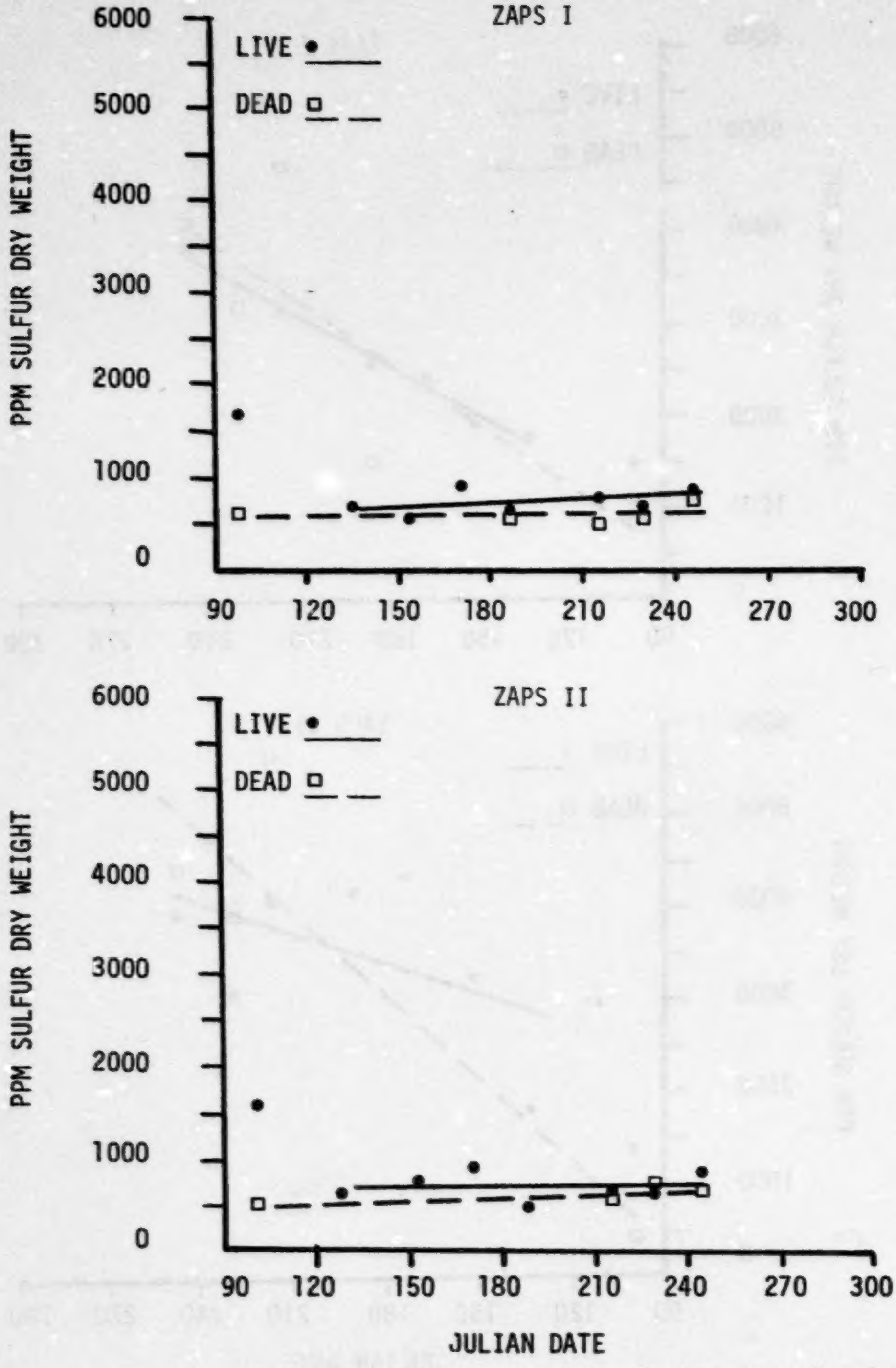

Figure 13.13. Linear regressions of sulfur levels through time in *Koeleria cristata* at CONTROL fumigation.

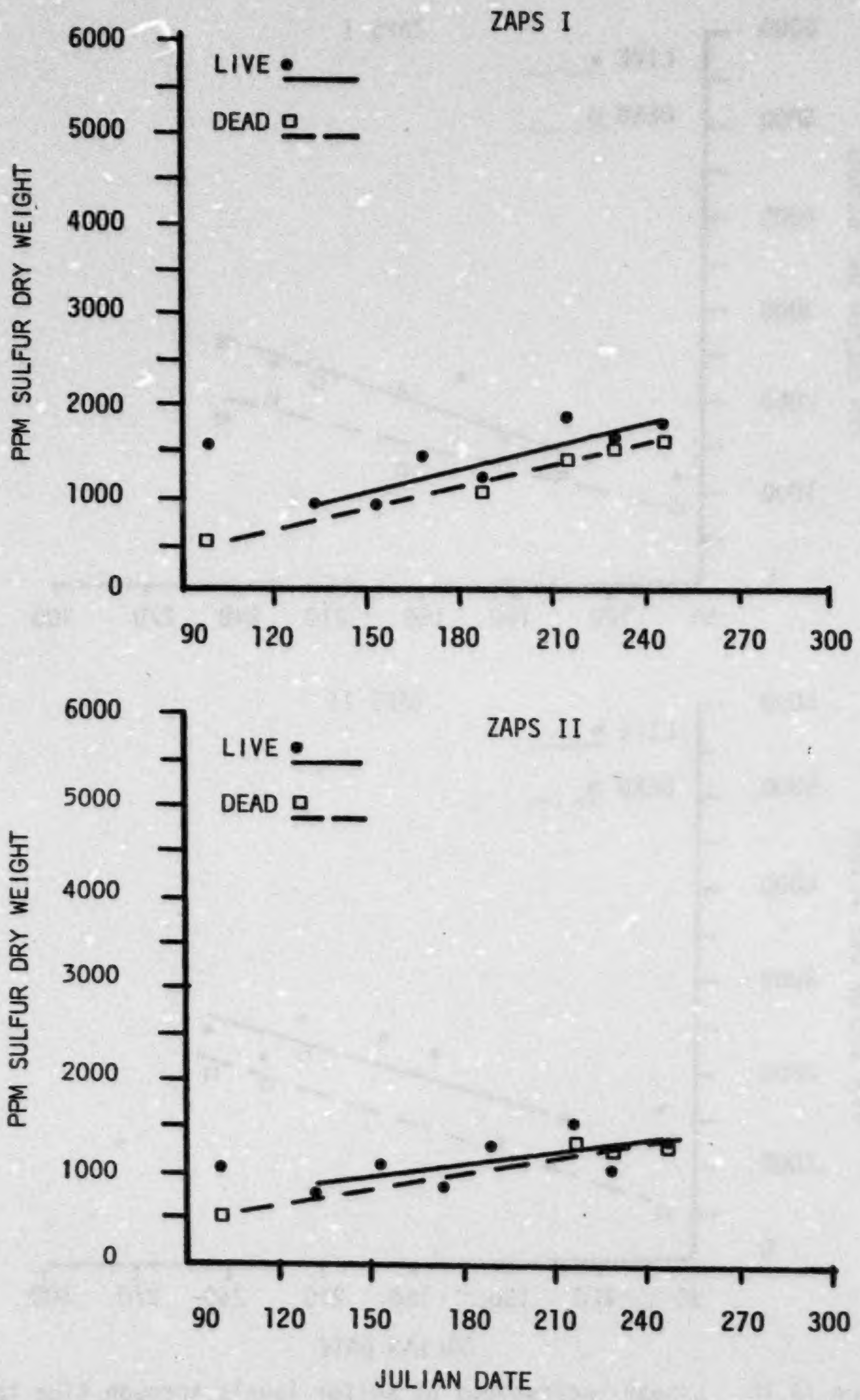

Figure 13.14. Linear regressions of sulfur levels through time in _Koeleria cristata_ at LOW fumigation.

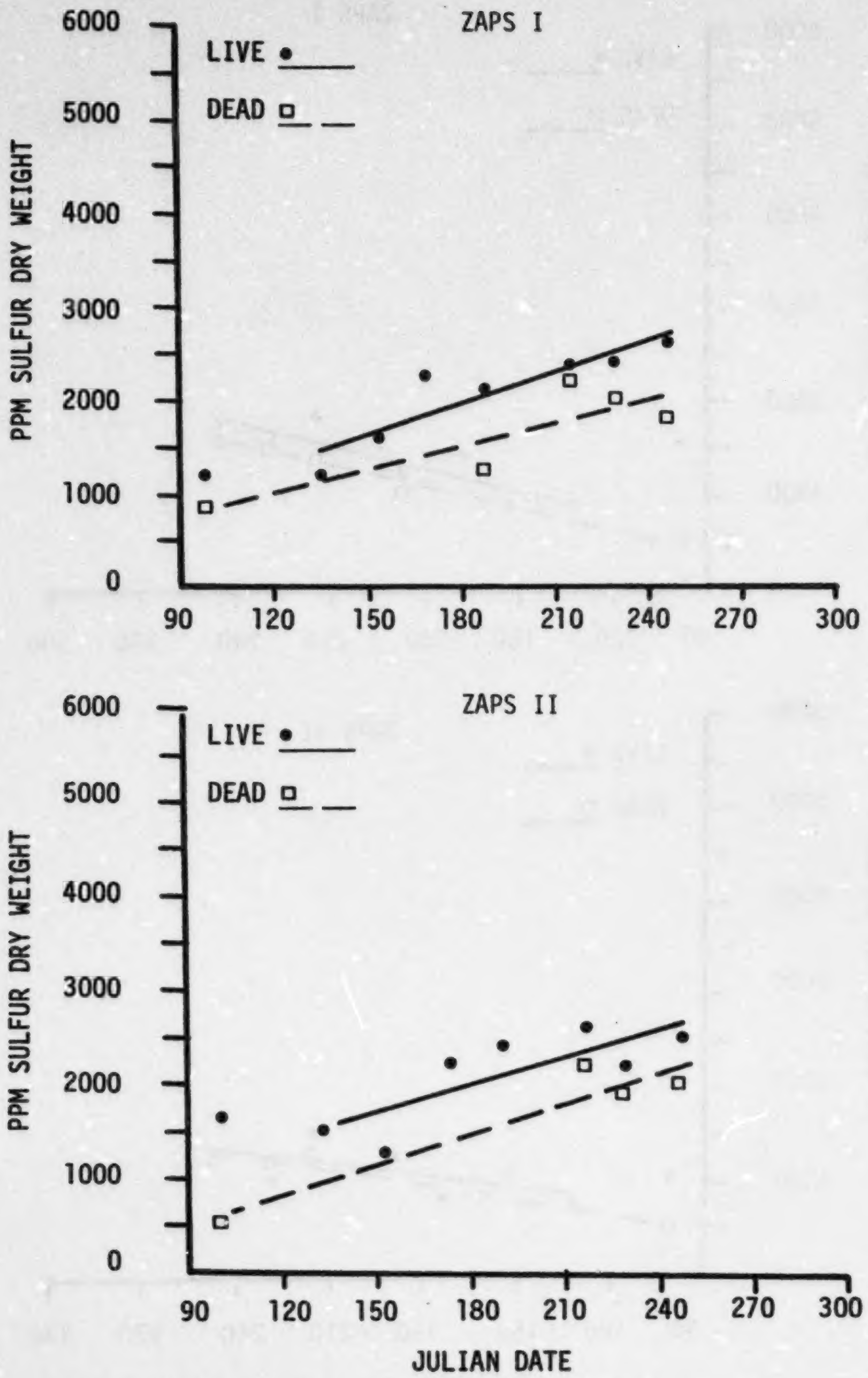

Figure 13.15. Linear regressions of sulfur levels through time in *Koeleria cristata* at MEDIUM fumigation.

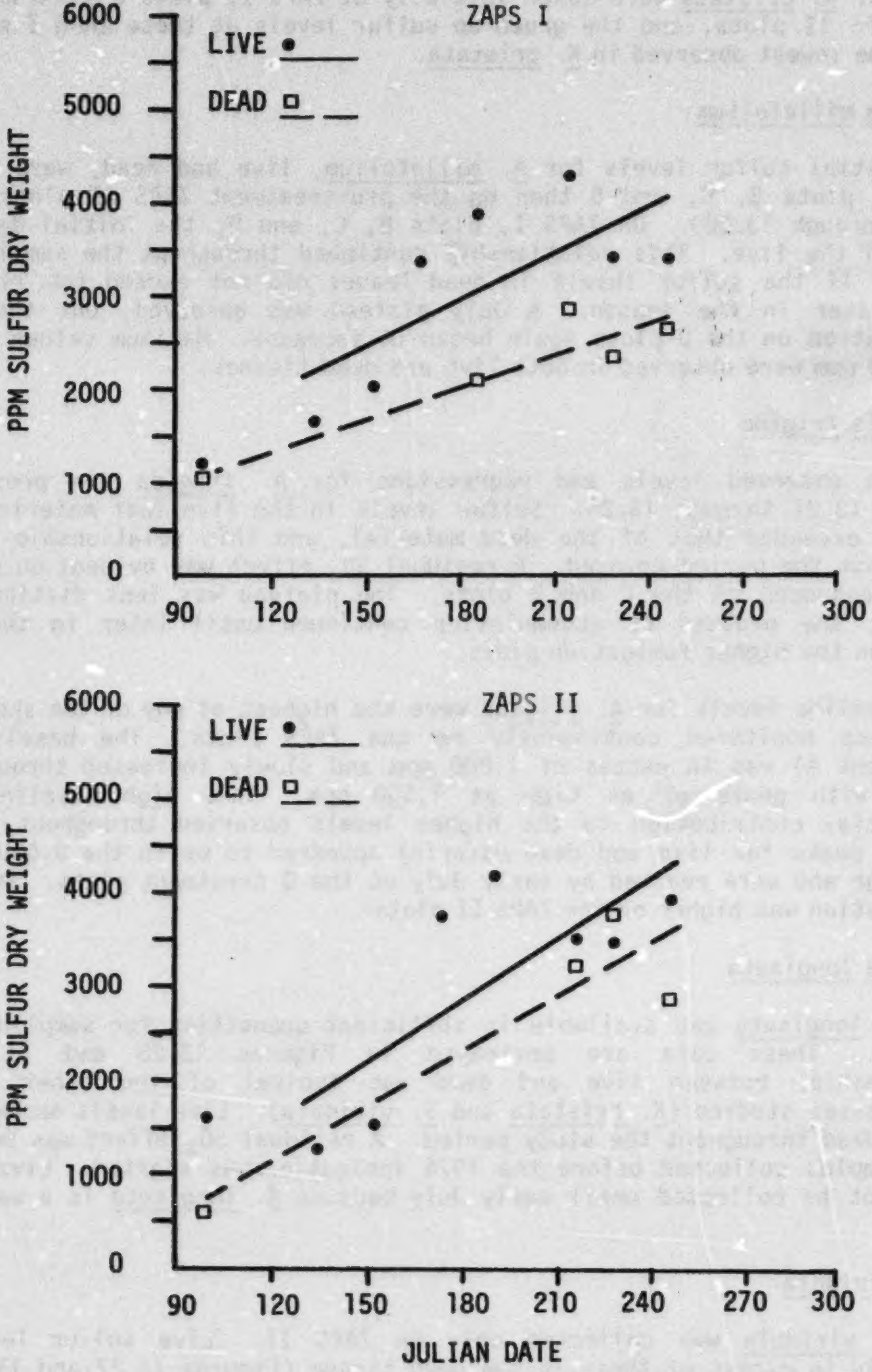

Figure 13.16. Linear regressions of sulfur levels through time in *Koeleria cristata* at HIGH fumigation.

Levels in K. cristata were lower initially at ZAPS I, plots C and D than at the same ZAPS II plots, and the green-up sulfur levels at these ZAPS I sites were among the lowest observed in K. cristata.

### Achillea millefolium

Initial sulfur levels for A. millefolium, live and dead, were higher on ZAPS I, plots B, C, and D than on the pre-treatment ZAPS II plots (Figures 13.17 through 13.20). On ZAPS I, plots B, C, and D, the initial dead levels exceeded the live. This relationship continued throughout the sample period. On ZAPS II the sulfur levels in dead leaves did not exceed the live leaves until later in the season. A July plateau was observed, but the rate of accumulation on the D plots again began to increase. Maximum values in excess of 6,000 ppm were observed in both live and dead tissues.

### Artemisia frigida

The observed levels and regressions for A. frigida are presented in Figures 13.21 through 13.24. Sulfur levels in the live leaf material of this species exceeded that of the dead material, and this relationship continued throughout the period covered. A residual $SO_2$ effect was evident on ZAPS I and very pronounced on the C and D plots. The plateau was less distinct for A. frigida; the process of accumulation continued until later in the growing season on the higher fumigation plots.

Baseline levels for A. frigida were the highest of any of the species that have been monitored continuously on the ZAPS plots. The baseline level (treatment A) was in excess of 1,000 ppm and slowly increased throughout the season with peaks of as high as 1,550 ppm. This high baseline made a substantial contribution to the higher levels observed throughout the year. Maximum peaks for live and dead material appeared to be in the 5,000 to 6,000 ppm range and were reached by early July on the D treatment plots. The rate of accumulation was higher on the ZAPS II plots.

### Aristida longiseta

A. longiseta was available in sufficient quantities for sampling only on ZAPS I. These data are portrayed in Figures 13.25 and 13.26. The relationship between live and dead was typical of the other perennial bunchgrasses studied (K. cristata and S. viridula). Live levels exceeded those of the dead throughout the study period. A residual $SO_2$ effect was seen in the dead samples collected before the 1976 fumigation was started. Live material could not be collected until early July because A. longiseta is a warm season grass.

### Stipa viridula

S. viridula was collected only on ZAPS II. Live sulfur levels were generally in excess of those in the dead tissue (Figures 13.27 and 13.28), but the dead approached the live at an increasing rate as the fumigation level increased. Live tissue reached a mid-July plateau on the plots under intentional fumigation. This was followed by a decline in sulfur until mid-

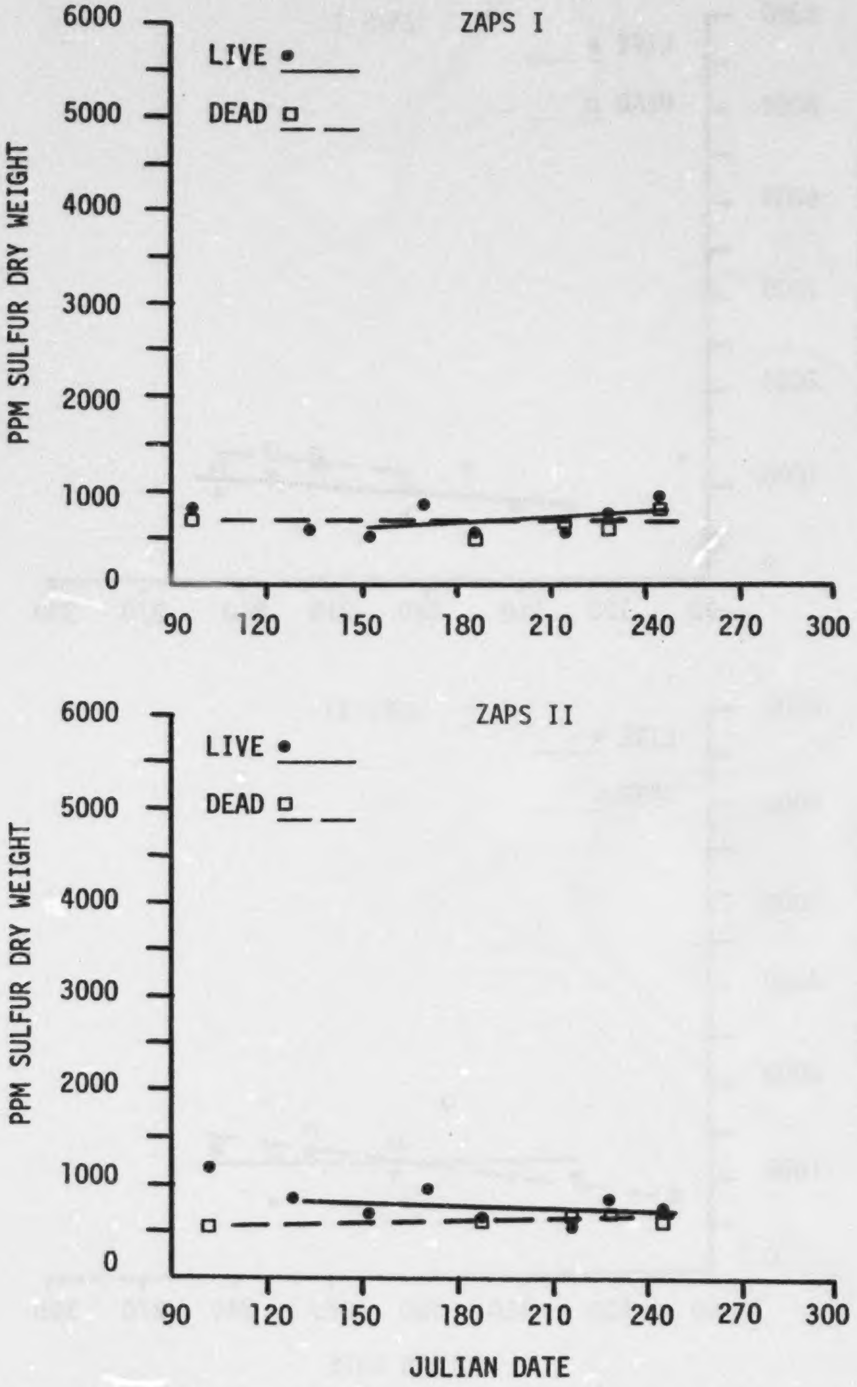

Figure 13.17. Linear regressions of sulfur levels through time in *Achillea millefolium* at CONTROL fumigation.

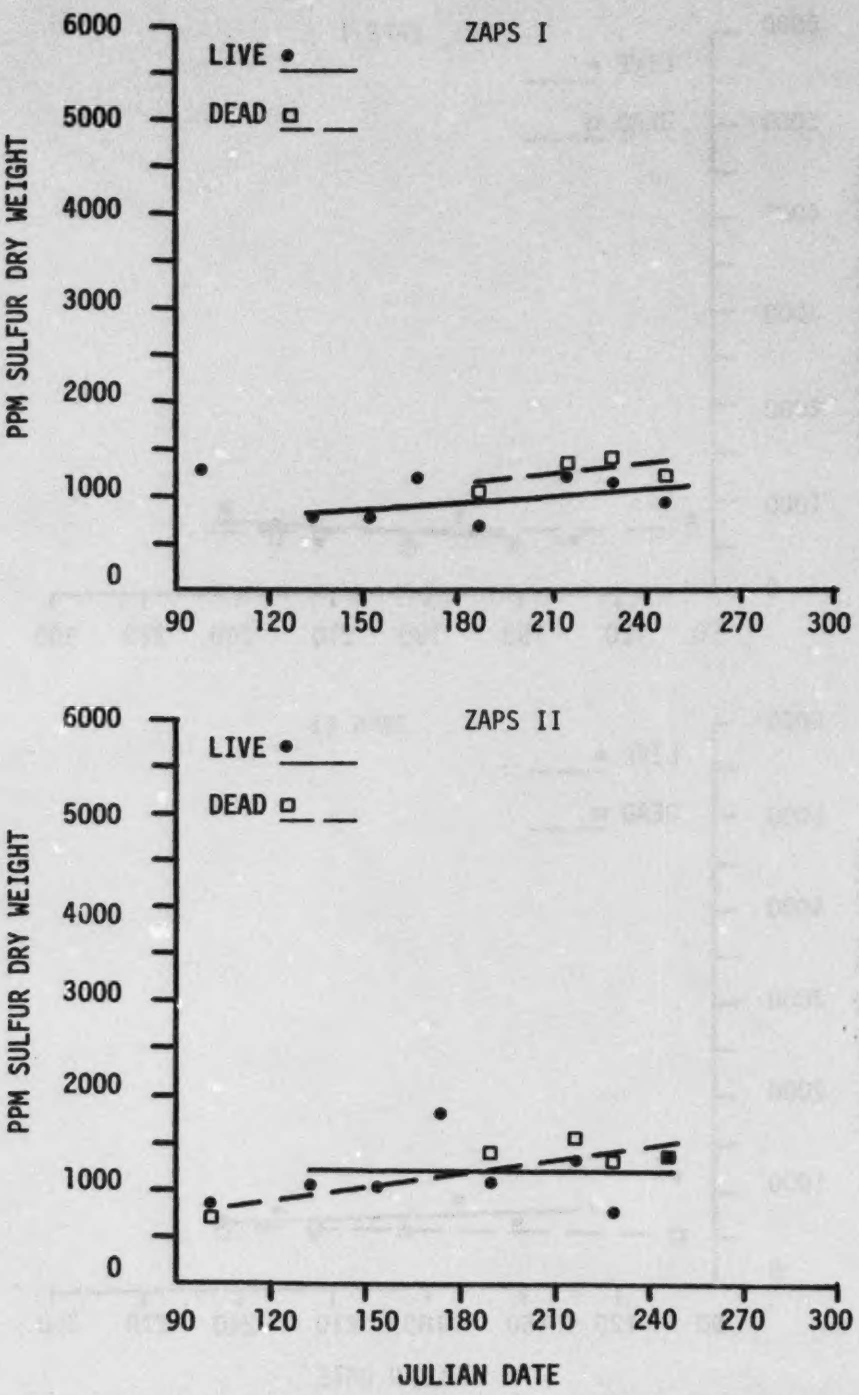

Figure 13.18. Linear regressions of sulfur levels through time in Achillea millefolium at LOW fumigation.

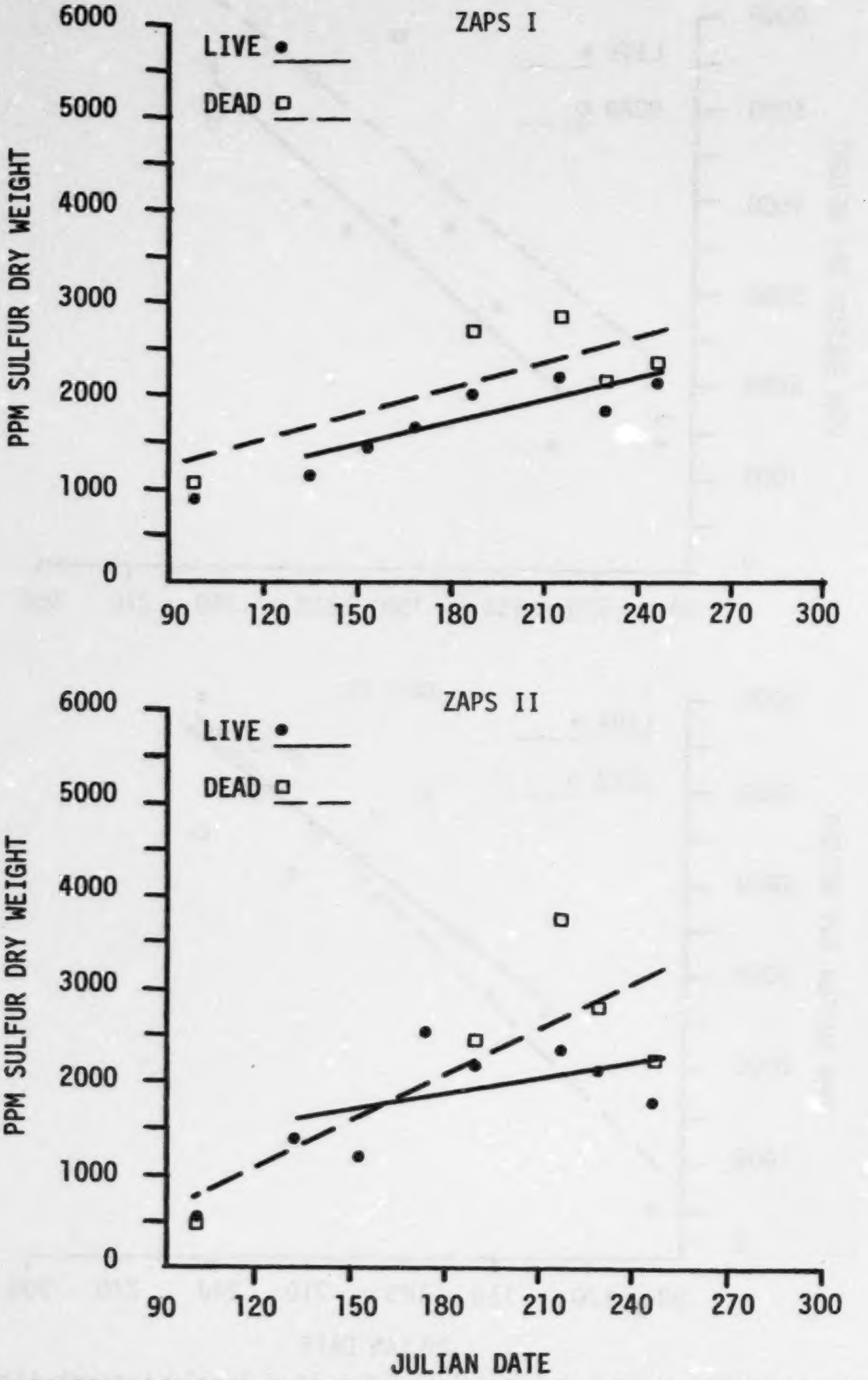

Figure 13.19. Linear regressions of sulfur levels through time in *Achillea millefolium* at MEDIUM fumigation.

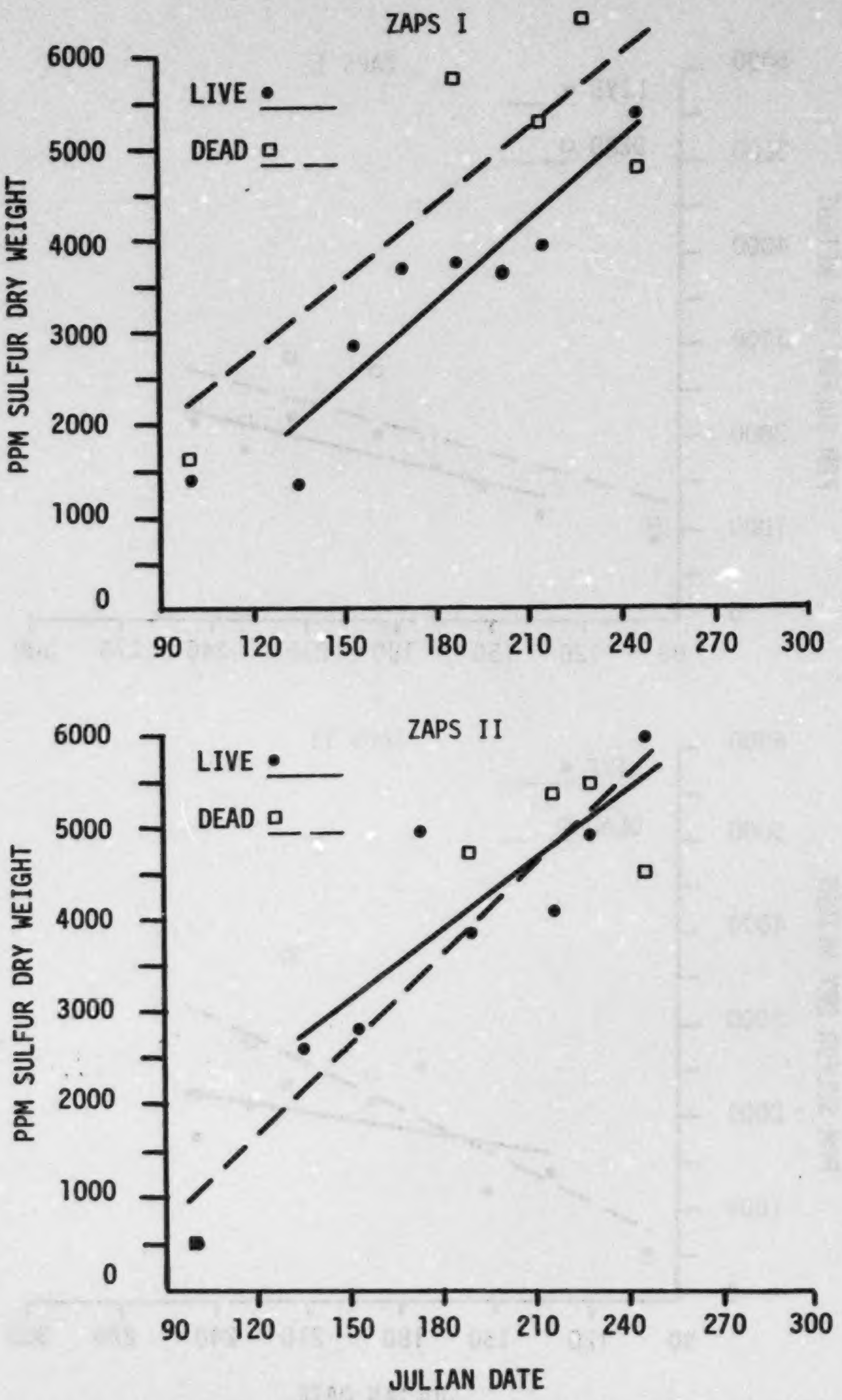

Figure 13.20. Linear regressions of sulfur levels through time in *Achillea millefolium* at HIGH fumigation.

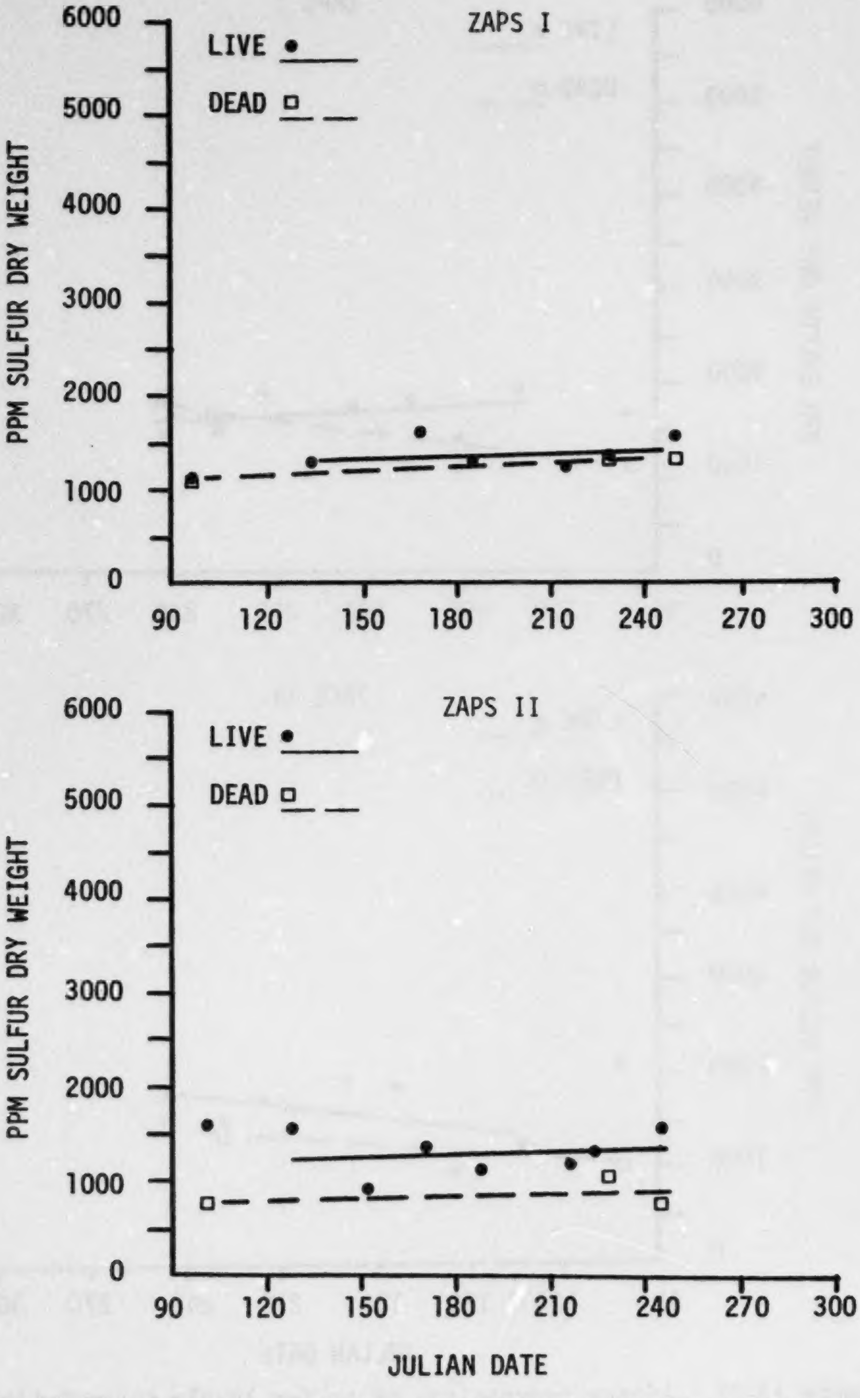

Figure 13.21. Linear regressions of sulfur levels through time in Artemisia frigida at CONTROL fumigation.

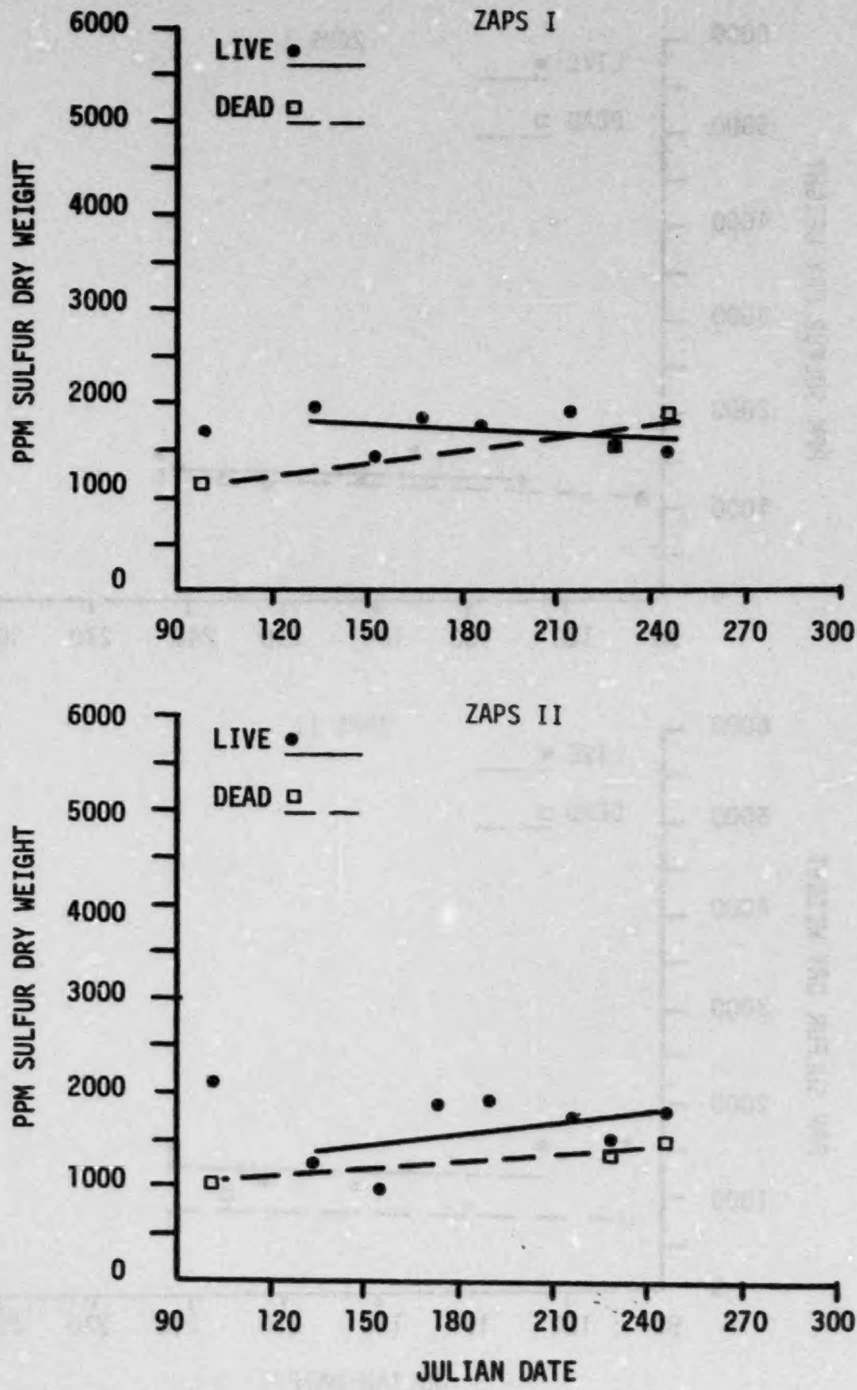

Figure 13.22. Linear regressions of sulfur levels through time in Artemisia frigida at LOW fumigation.

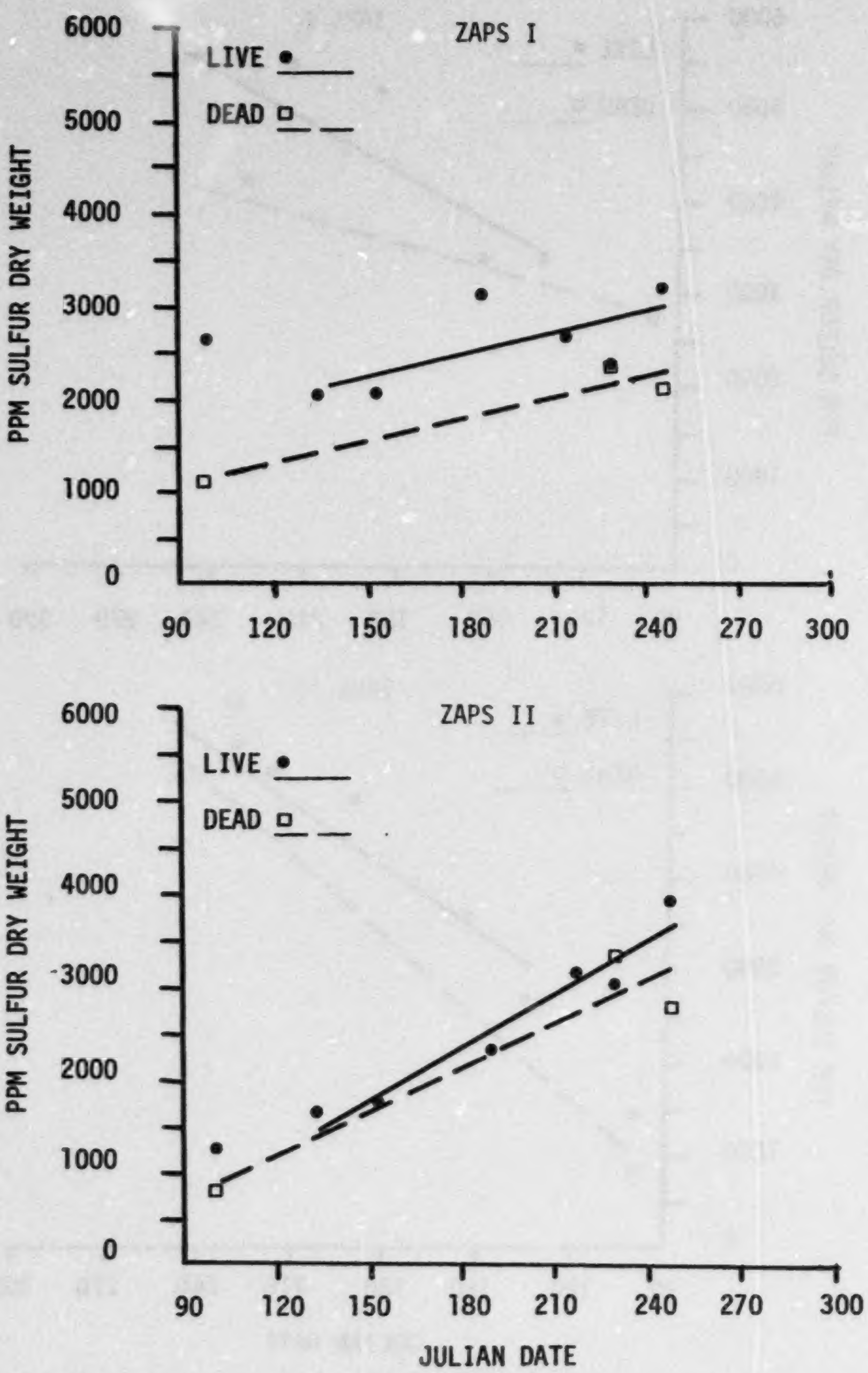

Figure 13.23. Linear regressions of sulfur levels through time in _Artemisia_ _frigida_ at MEDIUM fumigation.

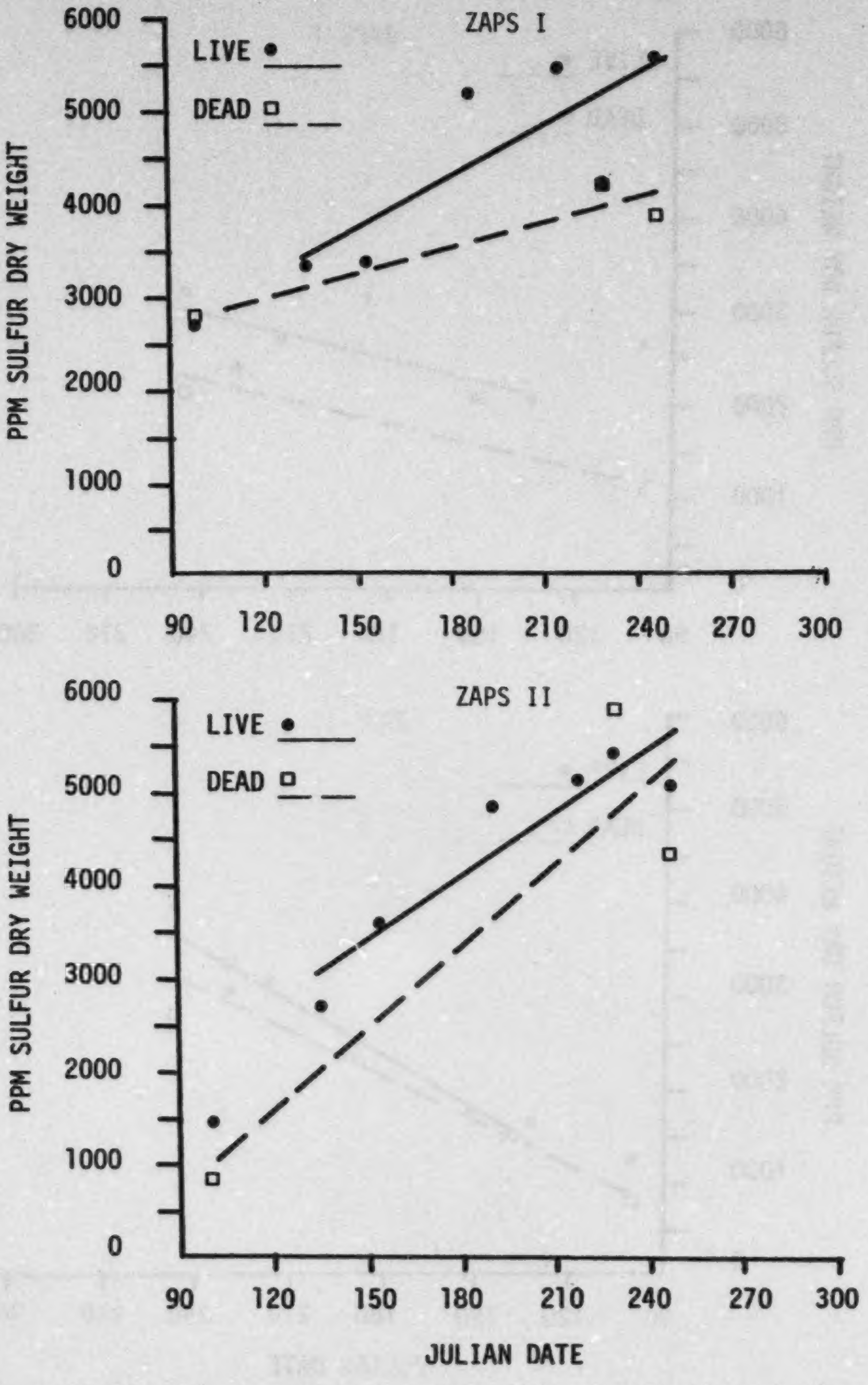

Figure 13.24. Linear regressions of sulfur levels through time in *Artemisia frigida* at HIGH fumigation.

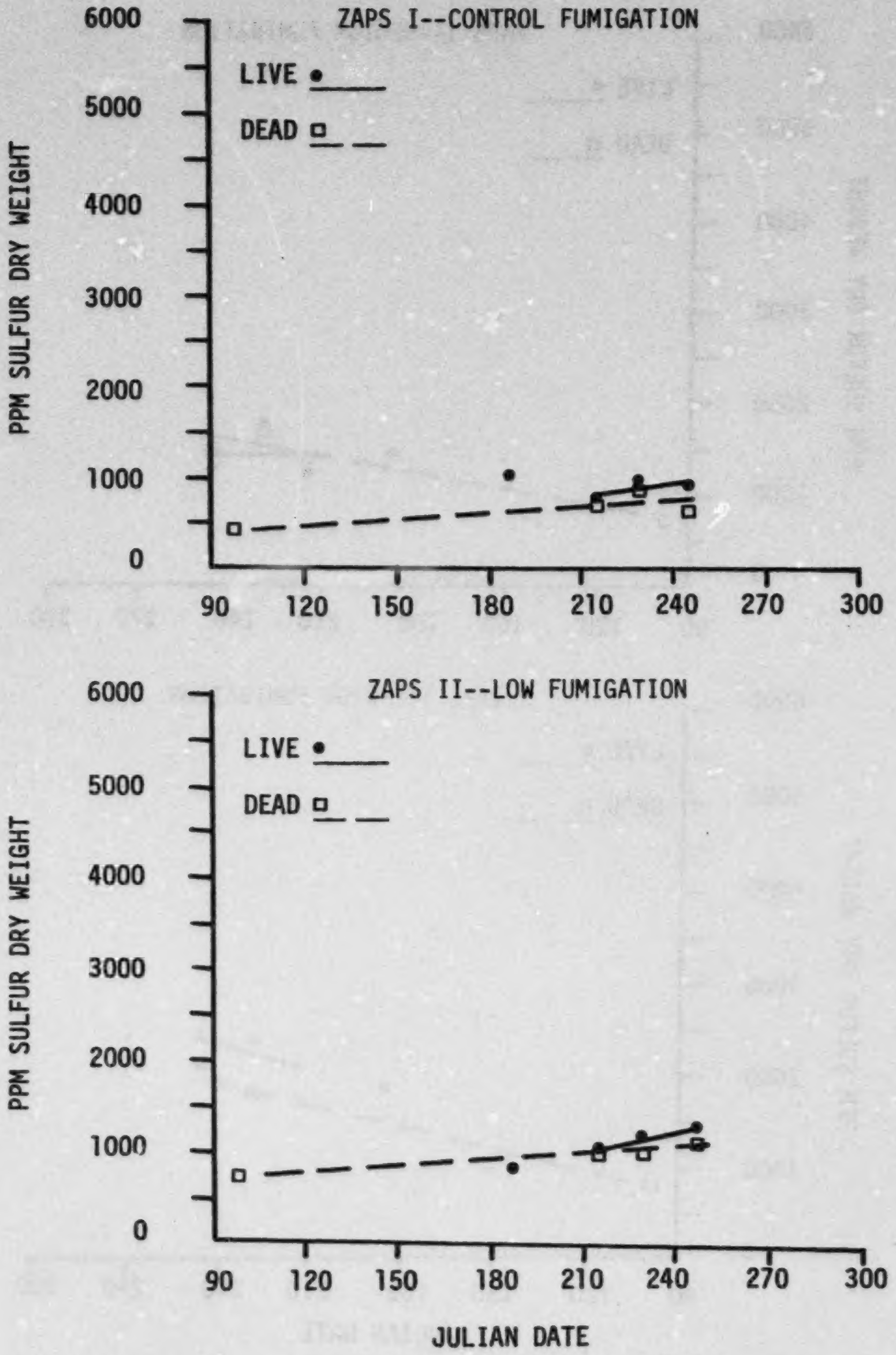

Figure 13.25. Linear regressions of sulfur levels through time in *Aristida longiseta* at CONTROL and LOW fumigations.

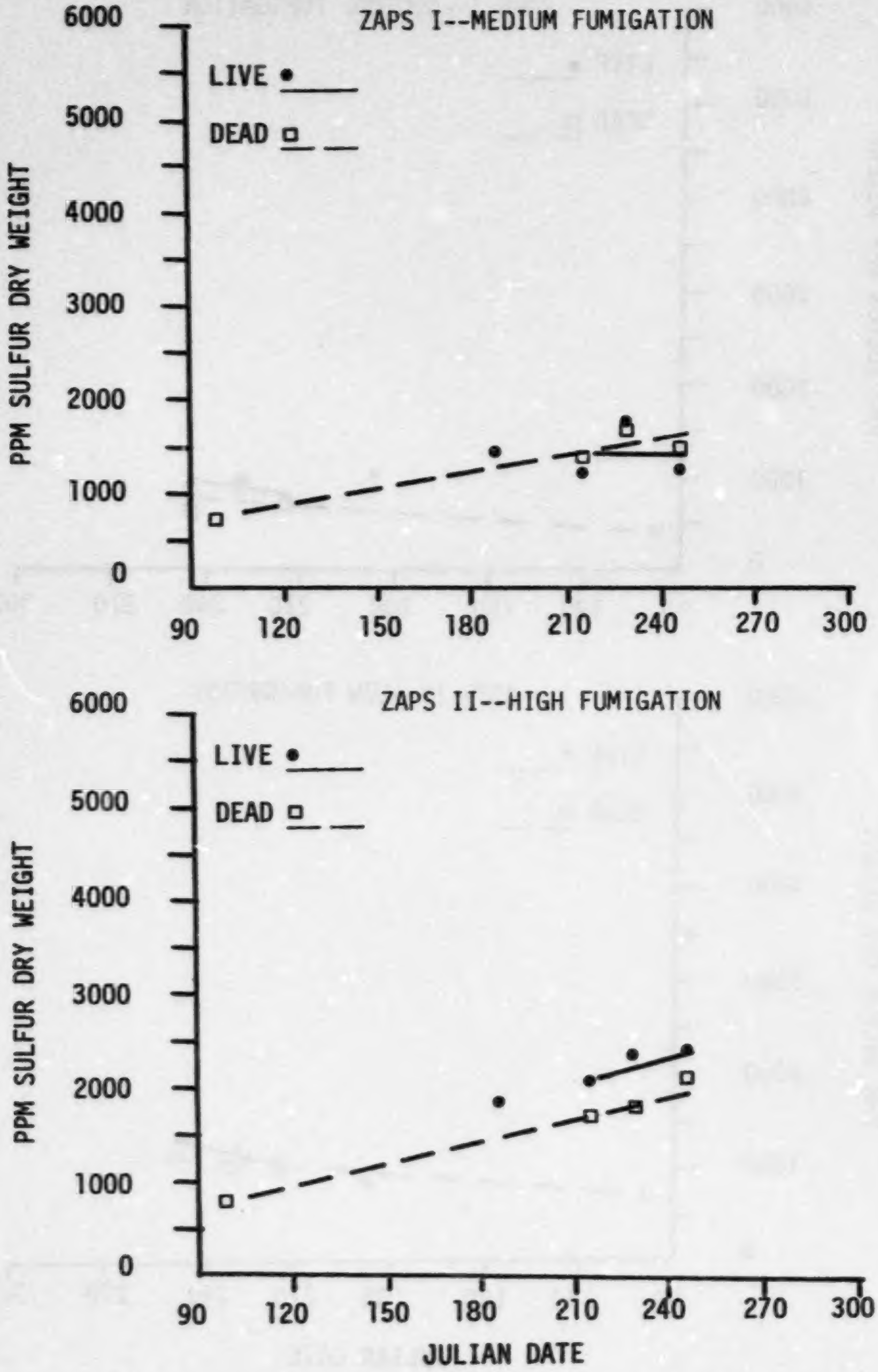

Figure 13.26. Linear regressions of sulfur levels through time in *Aristida longiseta* at MEDIUM and HIGH fumigations.

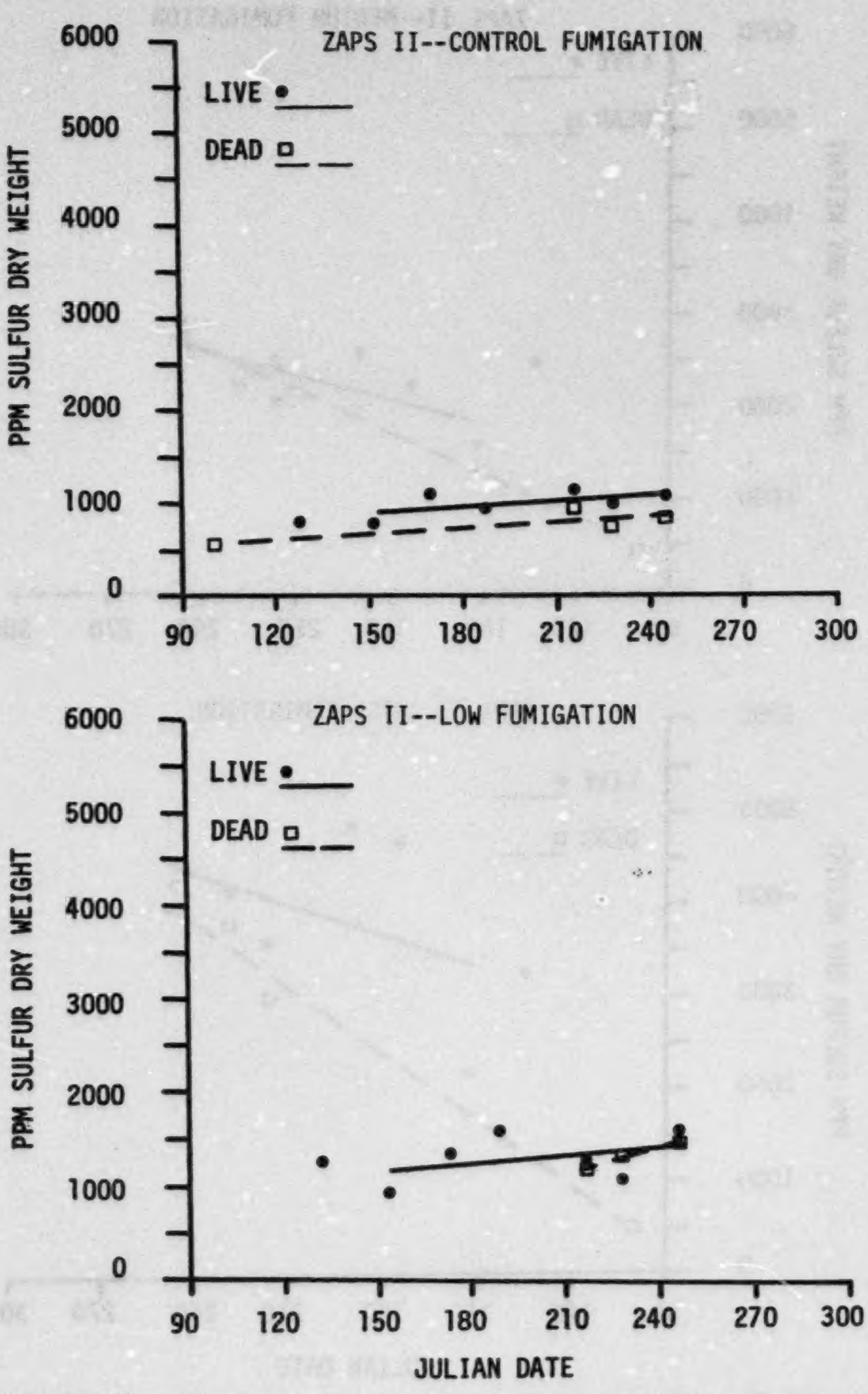

Figure 13.27. Linear regressions of sulfur levels through time in Stipa viridula at CONTROL and LOW fumigations.

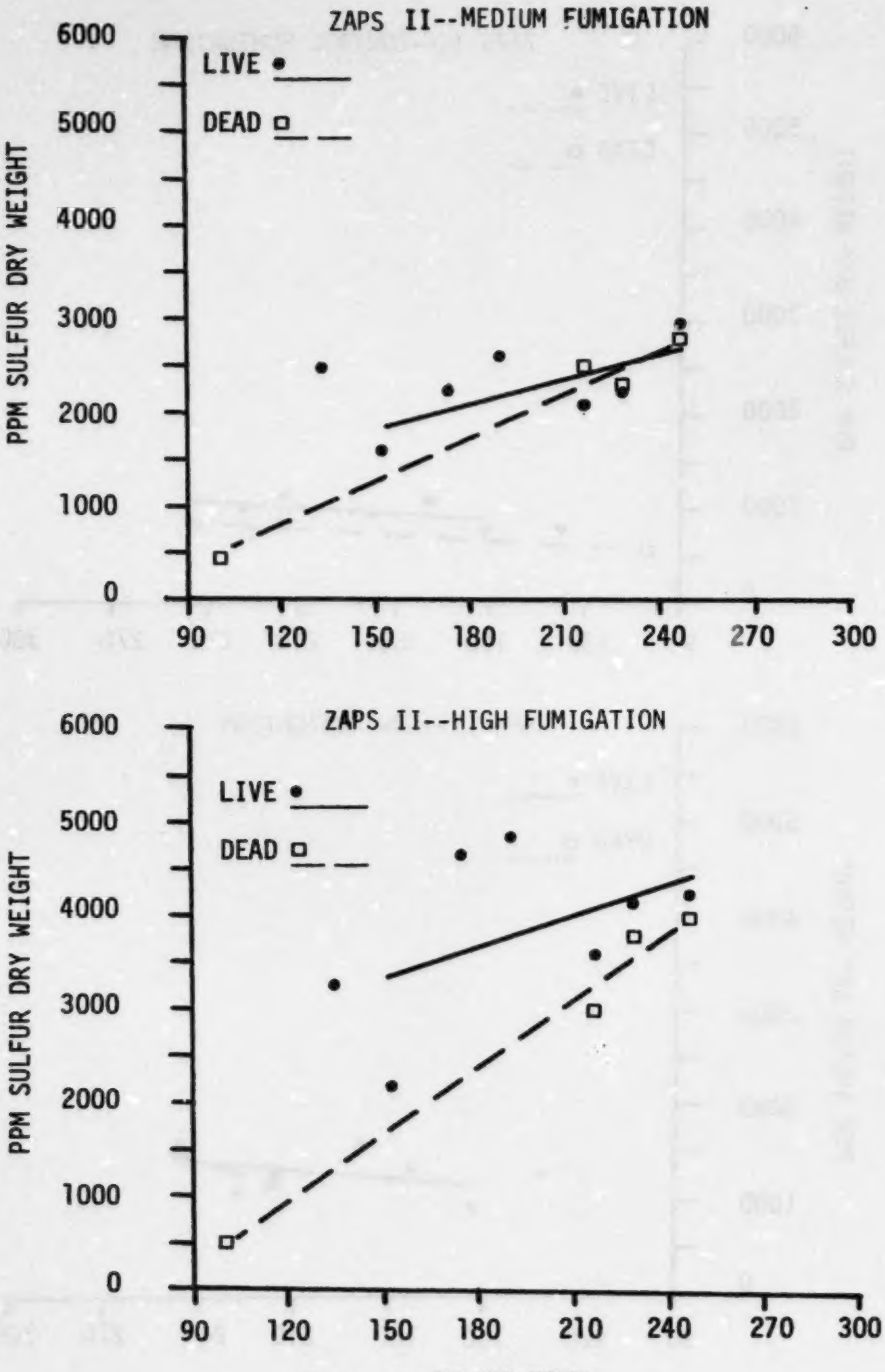

Figure 13.28. Linear regressions of sulfur levels through time in *Stipa viridula* at MEDIUM and HIGH fumigations.

August when the levels rose a second time. Maximum observed level in live foliage was 4,850 ppm at the D fumigation levels.

Poa sandbergii

The measured sulfur levels for P. sandbergii are graphed in Figures 13.29 through 13.32. Observed levels in both live and dead tissue increased with the fumigation level and tended to increase with time. The maximum level in both live and dead tissue was approximately 2,400 ppm. A limited number of data points precluded regression computation and interpretation of trends.

Tragopogon dubius

T. dubius was collected only in early July. The measured sulfur levels in the leaves, taproots, and stems are presented in Table 13.4. The small sample size precluded testing for significance. T. dubius showed increasing sulfur levels in all plant parts with higher levels of fumigation. Sulfur levels decreased in the following order: leaves, stems, taproots. The sulfur levels in this plant exceeded those in any others that have been studied by the investigators.

Roots

A. smithii roots collected in early April were improperly stored prior to preparation for sulfur analysis. Molding caused a large reduction in measurable sulfur levels. These data are not presented. Root collections of A. smithii and K. cristata made in early July were first tested for homogeneity of variances (Table 13.5). As the variances could be assumed equal, an anova was performed (Table 13.6). A significant difference in root sulfur levels across the treatment plots could not be detected for either species during early July.

A. smithii and K. cristata roots (ZAPS I and ZAPS II) from mid-September (September 17, 18, 19; Collection Period 9) displayed equal variances (Table 13.5). Anova indicated significant ($p \leq .05$) differences between means for individual species subjected to the different fumigation levels (Table 13.7). The observed differences in mean sulfur levels were inspected for significance ($p \leq .05$) by the Least Significant Difference (LSD) method (Table 13.8). A. smithii roots from ZAPS I, plot D had significantly more sulfur than roots from the other three treatments. On ZAPS II, plot D, sulfur in A. smithii roots also exceeded levels in roots from the other three treatments. Additionally, the sulfur content of roots from plot C was significantly greater than those from plot A. In the case of K. cristata, roots from ZAPS I, plot D, were significantly higher in sulfur content than roots from the other three treatment plots. Roots from treatment C also had significantly more sulfur than those from treatment plot B. K. cristata roots from ZAPS II, plot D, also had significantly higher sulfur content than those from plots A, B, and C.

Drift Areas

The results of the drift plot collections (ZAPS I) (including mid-September) are presented in Table 13.9. Several significant differences

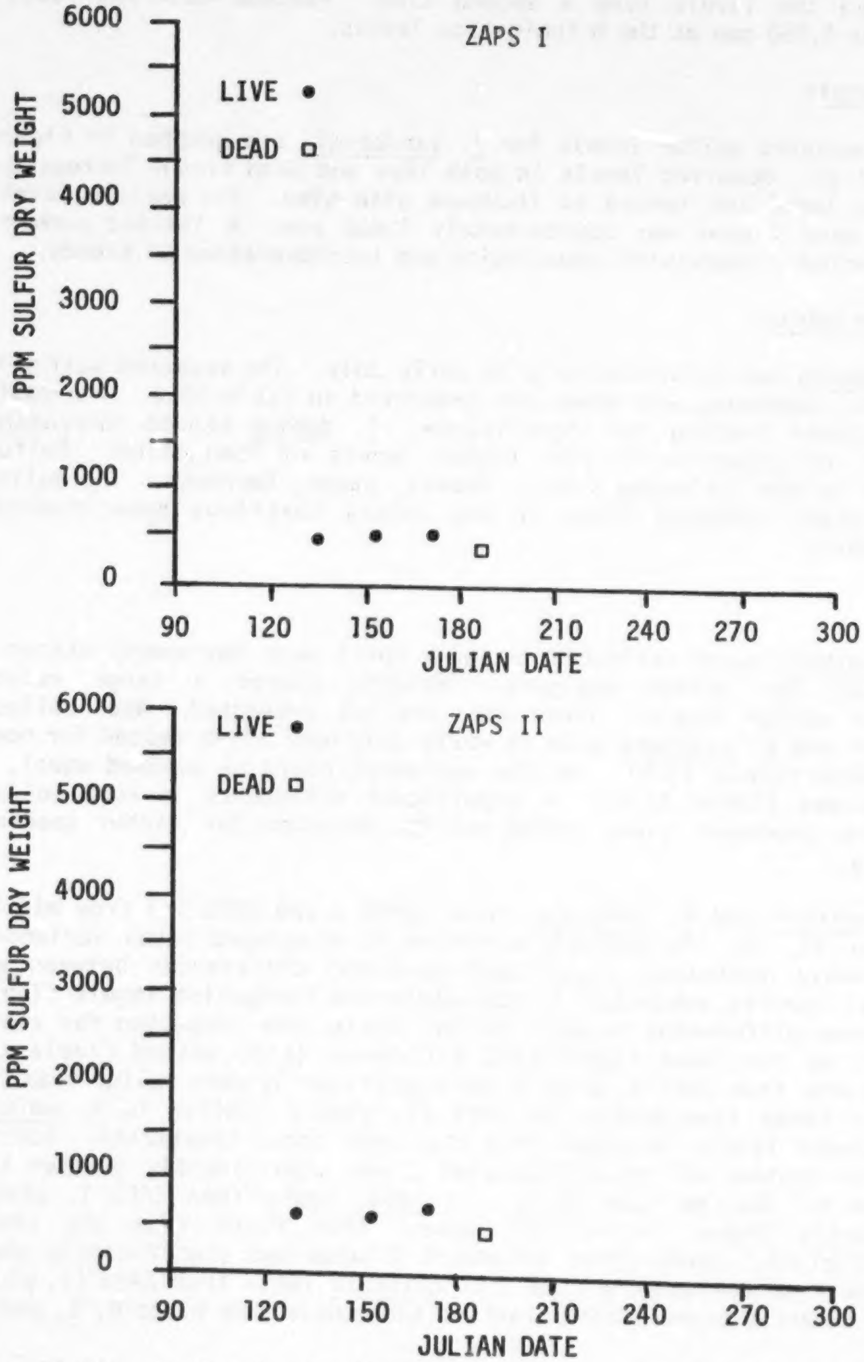

Figure 13.29. Sulfur levels through time in Poa sandbergii at CONTROL fumigation.

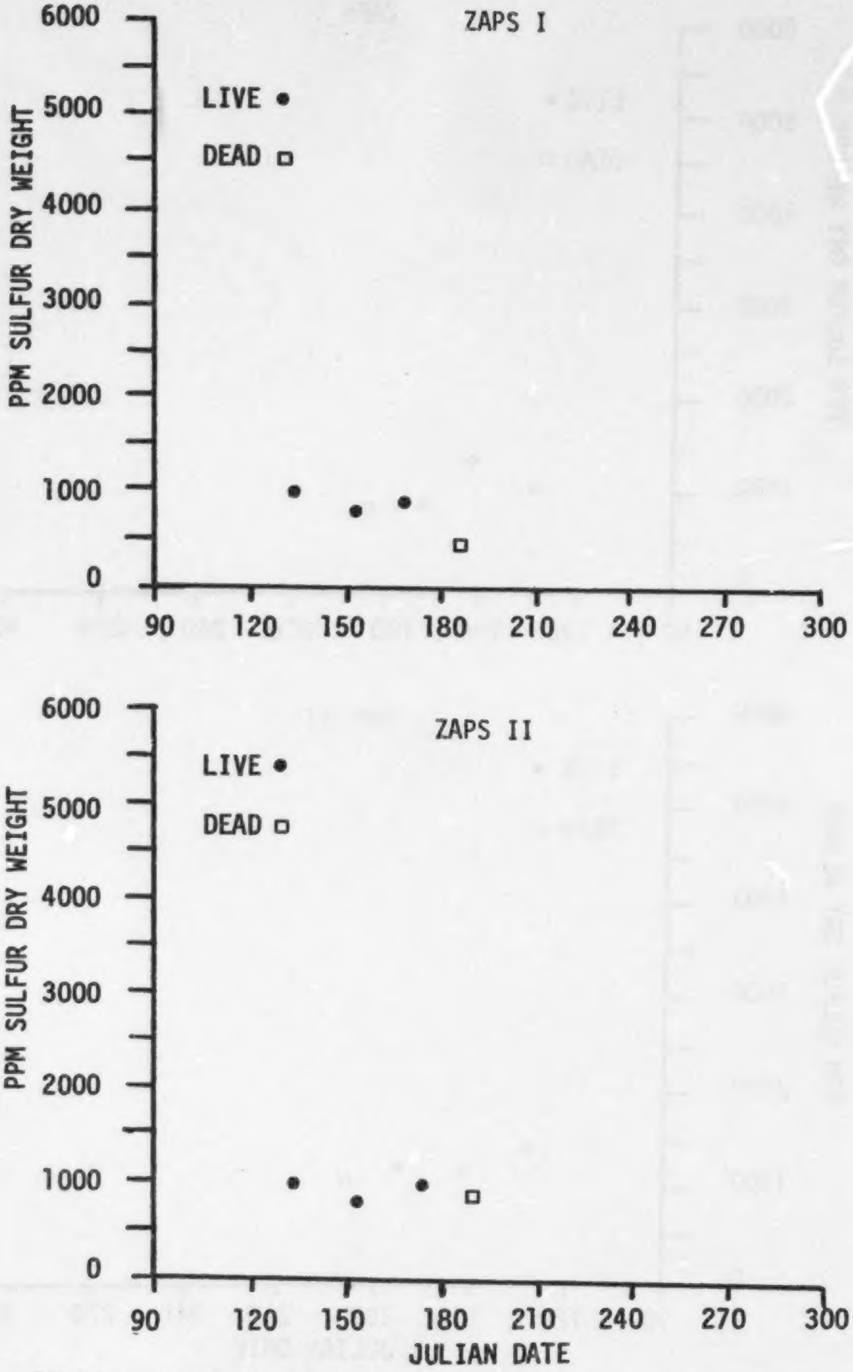

Figure 13.30. Sulfur levels through time in *Poa sandbergii* at LOW fumigation.

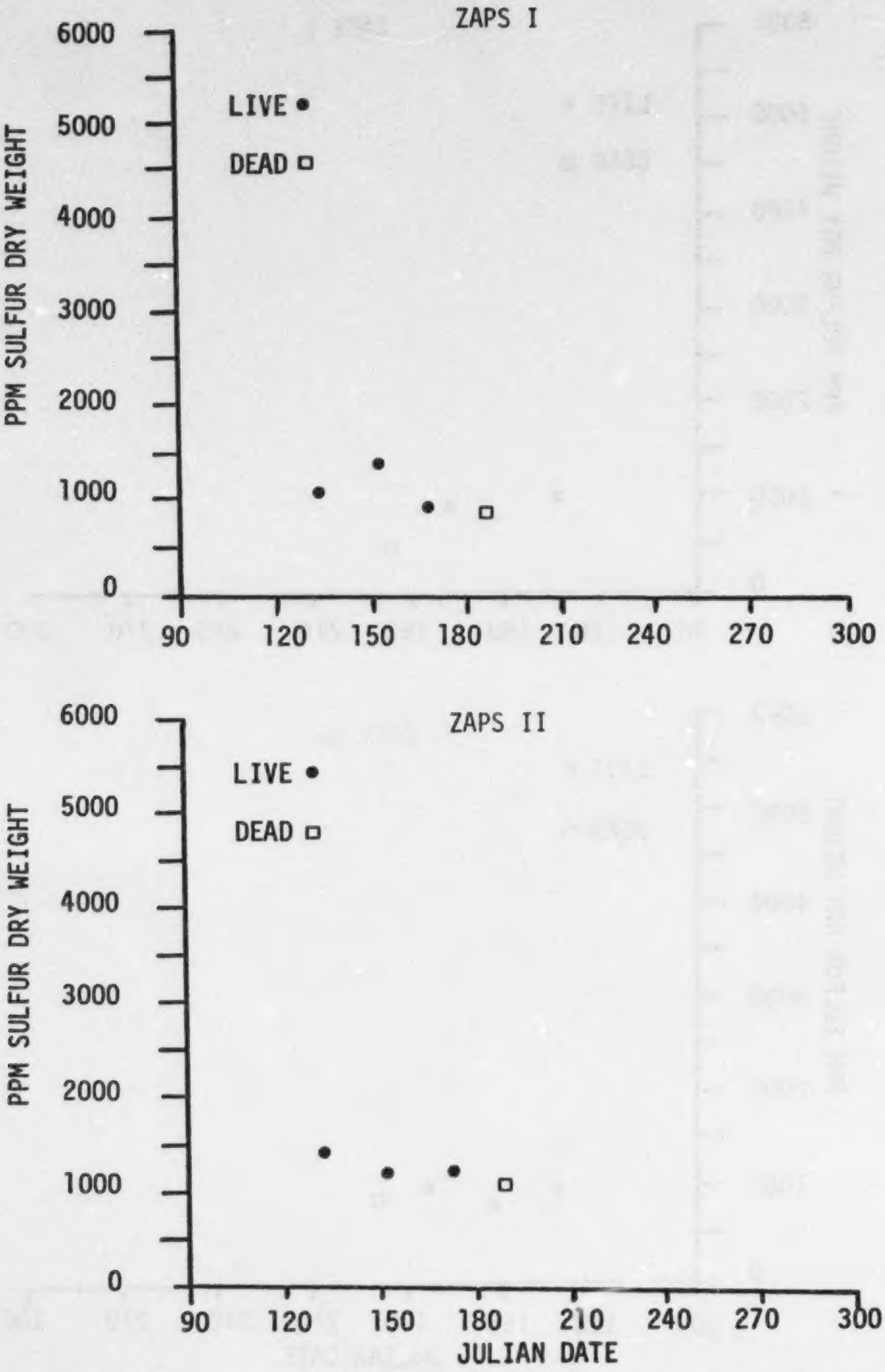

Figure 13.31. Sulfur levels through time in Poa sandbergii at MEDIUM fumigation.

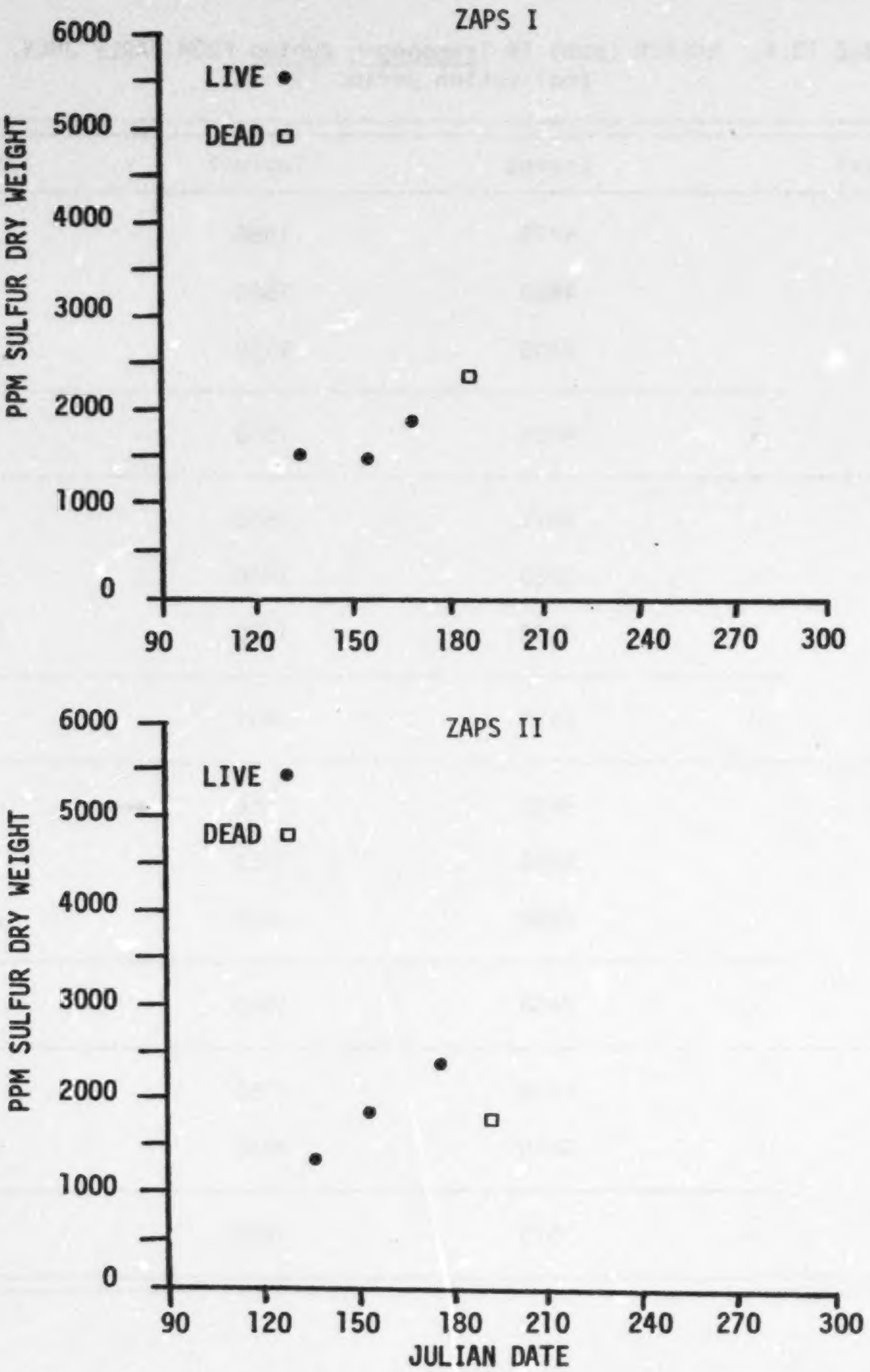

Figure 13.32. Sulfur levels through time in Poa sandbergii at HIGH fumigation.

TABLE 13.4. SULFUR (ppm) IN Tragopogon dubius FROM EARLY JULY. (collection period 5)

| Treatment | | Leaves | Taproot | Stem |
|---|---|---|---|---|
| Z1A | | 4775 | 1950 | - |
| | | 4450 | 1550 | - |
| | | 3100 | 1150 | 850 |
| | $\bar{x}$ | 4108 | 1550 | - |
| Z1B | | 6675 | 1550 | - |
| | | 5750 | 1900 | - |
| | | 7850 | 1400 | 1825 |
| | $\bar{x}$ | 7125 | 1617 | |
| Z1C | | 8625 | 1550 | - |
| | | 5750 | 1450 | - |
| | | 7850 | 1750 | 2000 |
| | $\bar{x}$ | 7408 | 1583 | |
| Z1D | | 5700 | 1150 | - |
| | | 9450 | 2450 | 3150 |
| | $\bar{x}$ | 7575 | 1800 | |

TABLE 13.5. MEAN SULFUR CONTENT AND BARTLETT'S TEST OF HOMOGENEITY OF VARIANCES FOR ROOTS FROM *A. Smithii* AND *K. Cristata*.

Collection Period 5, Early July, 1976

| | *A. smithii* | | | *K. cristata* | | |
|---|---|---|---|---|---|---|
| Treatment | $\bar{x}$ ppm | df=n-1 | $S^2$ | $\bar{x}$ ppm | df=n-1 | $S^2$ |
| Z1A | 780 | 9 | 9,565 | 792 | 9 | 6,115 |
| Z1B | 720 | 9 | 7,327 | 789 | 8 | 11,109 |
| Z1C | 790 | 9 | 6,006 | 805 | 9 | 6,368 |
| Z1D | 811 | 8 | 1,739 | 725 | 9 | 7,921 |
| | $\chi^2 = 5.28$ | | | $\chi^2 = 0.94$ | | |

Collection Period 9, Mid-September, 1976

| | *A. smithii* | | | *K. cristata* | | |
|---|---|---|---|---|---|---|
| Treatment | $\bar{x}$ ppm | df=n-1 | $S^2$ | $\bar{x}$ ppm | df=n-1 | $S^2$ |
| Z1A | 730 | 9 | 3,481 | 765 | 9 | 14,400 |
| Z1B | 738 | 9 | 6,889 | 725 | 9 | 6,241 |
| Z1C | 720 | 9 | 2,916 | 835 | 9 | 13,917 |
| Z1D | 830 | 9 | 6,241 | 970 | 9 | 7,921 |
| | $\chi^2 = 2.27$ | | | $\chi^2 = 2.15$ | | |
| Z2A | 720 | 9 | 1,764 | 738 | 8 | 14,884 |
| Z2B | 775 | 9 | 3,969 | 775 | 8 | 5,041 |
| Z2C | 790 | 9 | 6,561 | 825 | 8 | 6,400 |
| Z2D | 870 | 9 | 7,921 | 935 | 9 | 13,924 |
| | $\chi^2 = 4.92$ | | | $\chi^2 = 3.13$ | | |

Critical Chi-square $\chi^2$ .05 [3] = 7.82

TABLE 13.6. ANOVA OF SULFUR CONTENT OF ROOTS FOR Agropyron smithii AND Koeleria cristata FROM ZAPS I IN EARLY JULY. (collection period 5)

Agropyron smithii

| Source of Variation | Sum of Squares | Degrees of Freedom | Mean Square |
|---|---|---|---|
| Treatments | 44,470 | 3 | 14,823 |
| Residual | 219,889 | 35 | 6,283 |
| Total | 264,359 | 38 | |
| Critical $F_{.05[3,35]} = 2.875$ | | | $F_s = 2.359$ |

Koeleria cristata

| Source of Variation | Sum of Squares | Degrees of Freedom | Mean Square |
|---|---|---|---|
| Treatments | 38,542 | 3 | 12,847 |
| Residual | 272,451 | 35 | 7,784 |
| Total | 310,993 | 38 | |
| Critical $F_{.05[3,35]} = 2.875$ | | | $F_s = 1.650$ |

TABLE 13.7. ANOVA OF SULFUR CONTENT OF ROOTS FOR A. smithii AND K. cristata FROM ZAPS I AND ZAPS II IN MID-SEPTEMBER.
(collection period 9)

*Agropyron smithii* ZAPS I

| Source of Variation | Sum of Squares | Degrees of Freedom | Mean Square |
|---|---|---|---|
| Treatments | 77,797 | 3 | 25,932 |
| Residual | 174,562 | 36 | 4,849 |
| Total | 252,359 | 39 | |
| Critical $F_{.05[3,36]} = 2.88$ | | | $F_s = 5.348$ |

*Koeleria cristata* ZAPS I

| Source of Variation | Sum of Squares | Degrees of Freedom | Mean Square |
|---|---|---|---|
| Treatments | 382,750 | 3 | 127,583 |
| Residual | 347,188 | 36 | 9,644 |
| Total | 729,938 | 39 | |
| Critical $F_{.05[3,36]} = 2.88$ | | | $F_s = 10.885$ |

*Agropyron smithii* ZAPS II

| Source of Variation | Sum of Squares | Degrees of Freedom | Mean Square |
|---|---|---|---|
| Treatments | 115,188 | 3 | 38,396 |
| Residual | 182,250 | 36 | 5,063 |
| Total | 297,438 | 39 | |
| Critical $F_{.05[3,36]} = 2.88$ | | | $F_s = 7.584$ |

*Koeleria cristata* ZAPS II

| Source of Variation | Sum of Squares | Degrees of Freedom | Mean Square |
|---|---|---|---|
| Treatments | 202,250 | 3 | 67,417 |
| Residual | 309,000 | 30 | 10,300 |
| Total | 511,250 | 33 | |
| Critical $F_{.05[3,30]} = 2.92$ | | | $F_s = 6.545$ |

## TABLE 13.8. DIFFERENCES IN SULFUR CONTENT OF ROOTS COLLECTED IN MID-SEPTEMBER COMPARED BY LEAST SIGNIFICANT DIFFERENCES.

*Agropyron smithii* ZAPS I

| LSD .05<br>63 | 720<br>C | 730<br>A | 738<br>B | 830<br>D |
|---|---|---|---|---|
| 720<br>C | | | | |
| 730<br>A | 10 | | | |
| 738<br>B | 18 | 8 | | |
| 830<br>D | *<br>110 | *<br>100 | *<br>92 | |

*Koeleria cristata* ZAPS I

| LSD .05<br>90 | 725<br>B | 765<br>A | 835<br>C | 970<br>D |
|---|---|---|---|---|
| 725<br>B | | | | |
| 765<br>A | 40 | | | |
| 835<br>C | *<br>110 | 70 | | |
| 970<br>D | *<br>245 | *<br>205 | *<br>135 | |

*Agropyron smithii* ZAPS II

| LSD .05<br>65 | 720<br>A | 775<br>B | 790<br>C | 870<br>D |
|---|---|---|---|---|
| 720<br>A | | | | |
| 775<br>B | 55 | | | |
| 790<br>C | *<br>70 | 15 | | |
| 870<br>D | *<br>150 | *<br>95 | *<br>80 | |

*Koeleria cristata* ZAPS II

| LSD .05<br>104 | 738<br>A | 775<br>B | 825<br>C | 935<br>D |
|---|---|---|---|---|
| 738<br>A | | | | |
| 775<br>B | 47 | | | |
| 825<br>C | 87 | 50 | | |
| 935<br>D | *<br>197 | *<br>160 | *<br>110 | |

*Significant at $p \leq .05$

## TABLE 13.9. SULFUR LEVELS IN DRIFT PLOT COLLECTION.

| Collection Date | Julian Date | Species | Material | $\bar{X}$ | $S_{\bar{x}}$ | n | 95% Confidence Limits<br>$L_1$ | <br>$L_2$ |
|---|---|---|---|---|---|---|---|---|
| 13 April | 104 | *A. smithii* | Dead | 472 | 12 | 40 | 449 | 496 |
| 16 July | 198 | " | Live | 1004 | 36 | 34 | 933 | 1075 |
| " | " | " | Dead | 528 | 32 | 18 | 459 | 596 |
| " | " | " | Roots | 600 | 18 | 18 | 562 | 638 |
| " | " | *K. cristata* | Live | 802 | 44 | 20 | 711 | 894 |
| " | " | " | Dead | 612 | 31 | 19 | 546 | 678 |
| " | " | " | Roots | 752 | 22 | 20 | 705 | 800 |

($p \leq .05$) in partitioning of sulfur within the plants and between *A. smithii* and *K. cristata* were observed in the mid-July collection. *A. smithii* live tissue had more sulfur than either the dead tissue or the roots. *K. cristata* had more sulfur in the live tissue than in the dead. The level of sulfur in the dead tissue was less than that in the roots. Finally, live *A. smithii* contained more sulfur than *K. cristata*, while the dead tissue and roots of *A. smithii* contained less than did *K. cristata*.

A cluster pattern of differing sulfur levels within the drift areas was not established by the sampling grid used (Figure 13.5, Materials and Methods). The levels of sulfur by species in live culms for the entire mid-July drift collection were compared to the levels predicted by the A and B treatments on ZAPS I. Regression for both species at the LOW treatment level was significant and used to establish 95% prediction belts for sample sizes of 34 in the case of *A. smithi* and 20 in the case of *K. cristata*. The linear model for the A treatment (0 pphm) was rejected, and a prediction belt was constructed on $\hat{Y} = \bar{Y}$. The 95% confidence interval for the grasses from the drift plot area was then compared to the prediction belts for the appropriate point in time (July 16, i.e., day 198). This is portrayed graphically in Figure 13.33. The drift collections showed significantly lower levels in both species than in grasses fumigated on the B treatment plot. Although the means were higher than those from the plot A collections, they were not significantly so. No clusters on the drift areas were recognized as significantly higher than the plot A controls.

## PHENOLOGY

The results of the phenological observations of the vegetative stages of *A. smithii* are summarized in Tables 13.10 through 13.12. Total leaf number increased rapidly through early July and leveled off by the next observation period in early August (Tables 13.10 and 13.11). No significant differences ($p \leq .05$) in total leaf number were observed between the treatment plots at any observation date (Table 13.12).

The ratio of necrotic leaves to live leaves was examined to determine differences between treatments on each ZAPS plot during each observation period. The last row of Table 13.10 places the treatment plots on ZAPS I in order from that with the highest ratio of necrotic leaves to that with the lowest ratio of necrotic leaves for each observation period. The only significant ($p \leq .05$) difference observed was the September 2 collection in which vegetation from ZAPS I, plot D (HIGH) displayed a higher ratio of necrotic leaves than vegetation from ZAPS I, plot B (LOW). However, the trend from early July through mid-September was consistent, treatment plot D showing the highest proportion of necrotic leaves, followed by A (CONTROL), then C (MEDIUM), and finally B showing the smallest proportion of necrotic leaves. This relationship is portrayed graphically in Figures 13.34 through 13.36, where necrotic leaves (expressed as a percent of the total leaf number) of plot D are compared to plots A, B, and C. The exact probabilities (as opposed to $\alpha \leq .05$) of obtaining the observed differences on each date are presented in the next to the last row. The low probabilities from July on are indicative of the strength of the trend DACB. Only the HIGH treatment showed a higher level of necrosis than A (CONTROL).

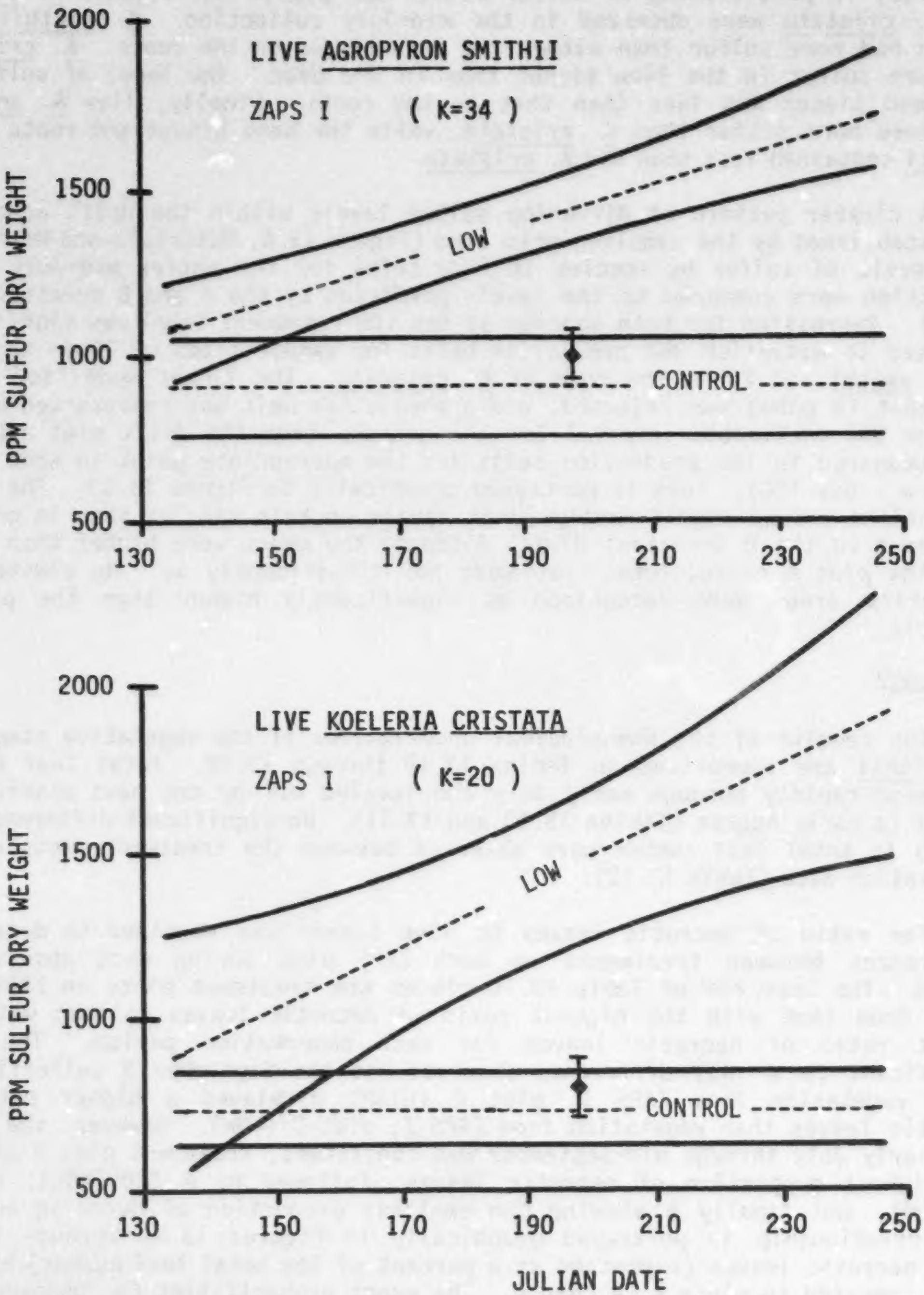

Figure 13.33. 95% prediction belts for treatment plots and 95% confidence interval ( ⧫ ) for drift plot collection.

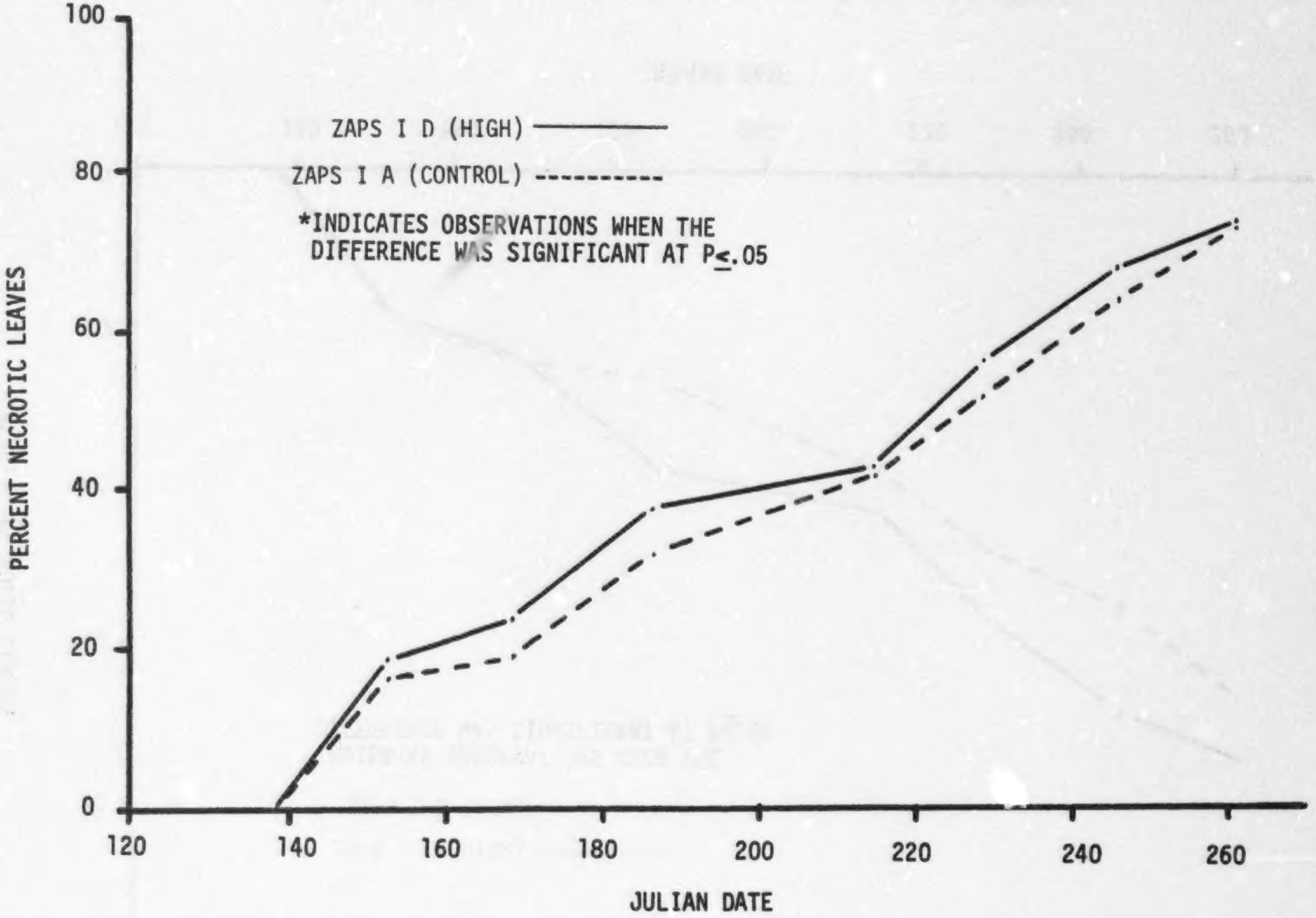

Figure 13.34. Percent necrotic leaves of Agropyron smithii.

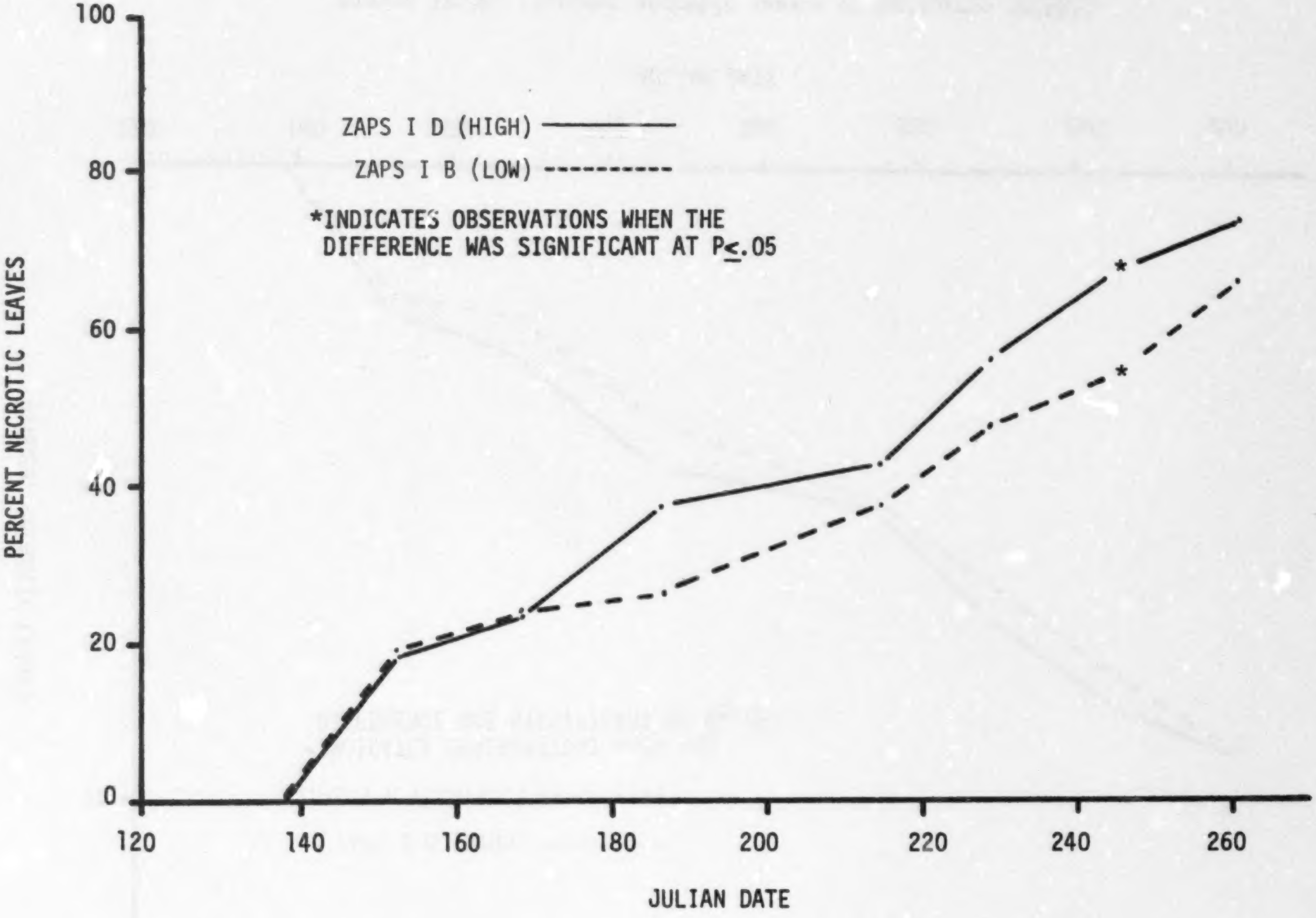

Figure 13.35. Percent necrotic leaves of Agropyron smithii.

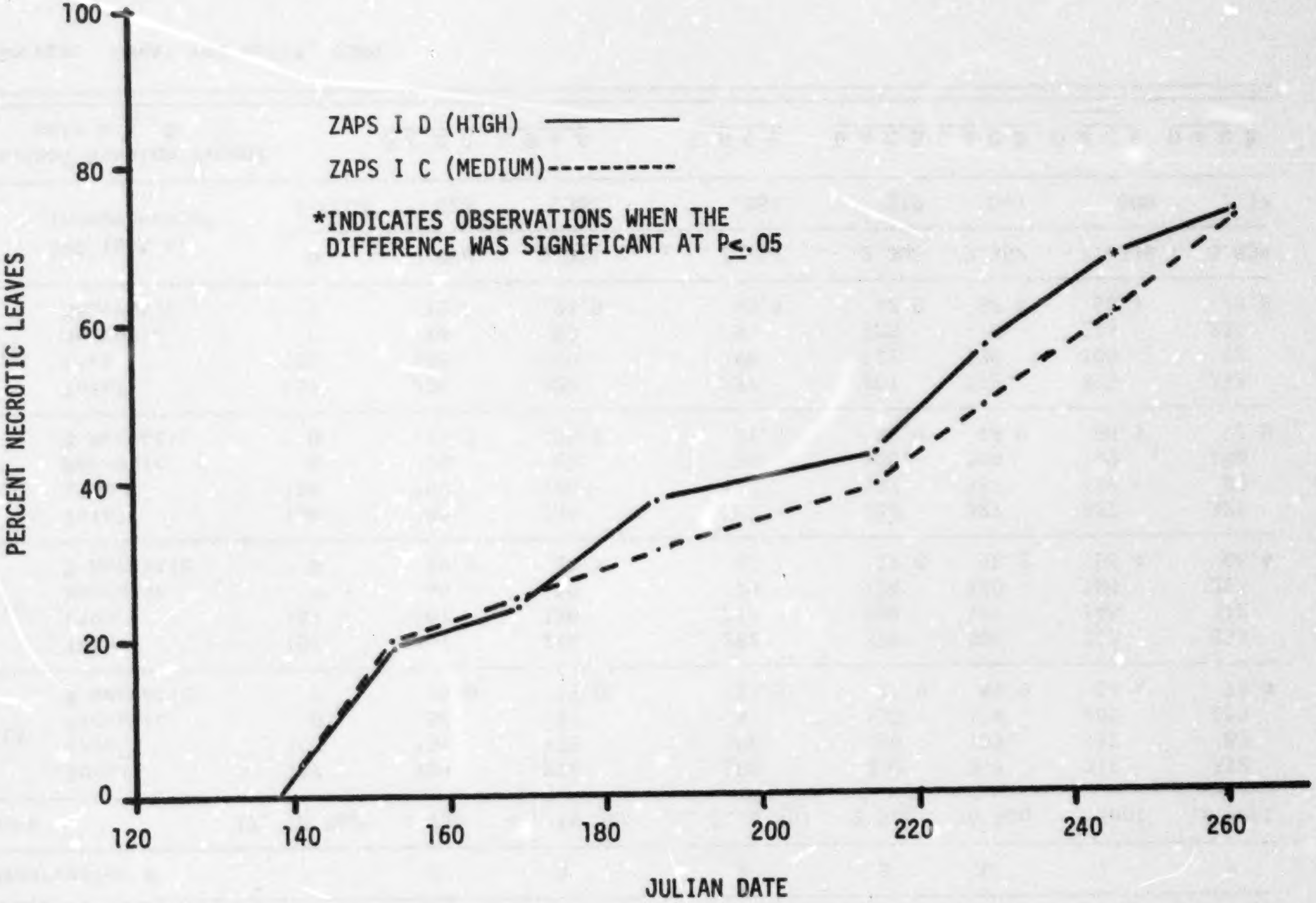

Figure 13.36. Percent necrotic leaves of Agropyron smithii.

TABLE 13.10. *Agropyron smithii* ZAPS I PHENOLOGY STUDIES AND TEST FOR INDEPENDENCE OF LEAF NECROSIS.

| Observation # | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|
| Date | | 17, 18 May | 1 Jun | 16, 18 Jun | 4, 5, 6 Jul | 2 Aug | 16 Aug | 2 Sept | 17 Sept |
| ZIA | Total | 147 | 193 | 216 | 288 | 310 | 311 | 312 | 312 |
| | Live | 147 | 161 | 175 | 196 | 180 | 137 | 112 | 83 |
| | Necrotic | 0 | 32 | 41 | 92 | 130 | 174 | 200 | 229 |
| | % Necrotic | 0 | 16.6 | 19.0 | 31.9 | 41.9 | 55.9 | 64.1 | 73.4 |
| ZIB | Total | 157 | 201 | 246 | 292 | 328 | 331 | 332 | 333 |
| | Live | 157 | 161 | 186 | 213 | 204 | 171 | 148 | 112 |
| | Necrotic | 0 | 40 | 60 | 79 | 124 | 160 | 184 | 221 |
| | % Necrotic | 0 | 19.9 | 24.4 | 27.1 | 37.8 | 48.3 | 55.4 | 66.4 |
| ZIC | Total | 154 | 198 | 234 | 259 | 322 | 327 | 327 | 329 |
| | Live | 154 | 159 | 175 | 178 | 197 | 167 | 128 | 89 |
| | Necrotic | 0 | 39 | 59 | 81 | 125 | 160 | 199 | 240 |
| | % Necrotic | 0 | 19.7 | 25.2 | 31.3 | 38.8 | 48.9 | 60.9 | 72.9 |
| ZID | Total | 151 | 200 | 222 | 237 | 301 | 312 | 313 | 313 |
| | Live | 151 | 162 | 169 | 146 | 172 | 136 | 100 | 82 |
| | Necrotic | 0 | 38 | 53 | 91 | 129 | 176 | 213 | 231 |
| | %Necrotic | 0 | 19.0 | 23.9 | 38.4 | 42.9 | 56.4 | 68.1 | 73.8 |
| RXC Test of Independence* | | G | 0.908 | 3.031 | 7.757 | 2.305 | 7.352 | 11.736 | 5.894 |
| | | 1-P(G) | .823 | .387 | .051 | .512 | .061 | .008 | .117 |
| Maximal Nonsignificant sets $\alpha \leq .05$ | | | B C D A | C B D A | D A C B | D A C B | D A C B | D A C B | D A C B |

*Source: Sokal and Rohlf, 1969.

TABLE 13.11. Agropyron smithii ZAPS II PHENOLOGY STUDIES AND TEST FOR INDEPENDENCE OF LEAF NECROSIS.

| Observation # | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|
| Date | | 23, 24 May | No Sample | 18, 21 Jun | 6, 7 Jul | 3 Aug | 15 Aug | 2 Sept | 19 Sept |
| Z2A | Total | 159 | | 201 | 275 | 281 | 281 | 285 | 286 |
| | Live | 159 | | 153 | 201 | 151 | 137 | 90 | 45 |
| | Necrotic | 0 | | 48 | 74 | 130 | 144 | 195 | 241 |
| | % Necrotic | 0 | | 23.9 | 26.9 | 46.3 | 51.2 | 68.4 | 84.3 |
| Z2B | Total | 158 | | 230 | 280 | 292 | 296 | 302 | 303 |
| | Live | 158 | | 184 | 213 | 158 | 143 | 104 | 64 |
| | Necrotic | 0 | | 46 | 67 | 134 | 153 | 198 | 239 |
| | % Necrotic | 0 | | 20.0 | 23.9 | 45.9 | 51.7 | 65.6 | 78.9 |
| Z2C | Total | 164 | | 230 | 294 | 304 | 307 | 307 | 308 |
| | Live | 164 | | 174 | 201 | 161 | 148 | 102 | 68 |
| | Necrotic | 0 | | 56 | 93 | 143 | 159 | 205 | 240 |
| | % Necrotic | 0 | | 24.3 | 31.6 | 47.0 | 51.8 | 66.8 | 77.9 |
| Z2D | Total | 145 | | 220 | 289 | 316 | 319 | 321 | 323 |
| | Live | 145 | | 150 | 170 | 127 | 124 | 88 | 57 |
| | Necrotic | 0 | | 76 | 119 | 189 | 195 | 233 | 266 |
| | % Necrotic | 0 | | 31.8 | 41.2 | 59.8 | 61.1 | 72.6 | 82.4 |
| RXC Test of Independence* | | G | | 8.578 | 22.444 | 16.855 | 8.686 | 4.149 | 5.130 |
| | | 1-P(G) | | .035 | .001 | .001 | .034 | .246 | .163 |
| Maximal Nonsignificant Sets $\alpha \leq .05$ | | | | D C A B | D C A B | D C A B | D C B A | D A C B | A D B C |

*Source: Sokal and Rohlf, 1969.

TABLE 13.12. CHI-SQUARE TEST OF TOTAL LEAF NUMBER AT EACH OBSERVATION.

| Collection Number | ZAPS I | | ZAPS II | |
|---|---|---|---|---|
| | $\chi^2$ | E | $\chi^2$ | E |
| 1 | 0.360 | 152 | 1.259 | 156 |
| 2 | 0.192 | 198 | No Sample | |
| 3 | 2.314 | 230 | 2.546 | 220 |
| 4 | 7.487 | 269 | 0.777 | 285 |
| 5 | 1.392 | 315 | 2.296 | 298 |
| 6 | 0.982 | 320 | 2.609 | 301 |
| 7 | 0.941 | 321 | 2.182 | 304 |
| 8 | 1.090 | 322 | 2.289 | 305 |

$\chi^2.05[3] = 7.815$; See Tables 13.10 and 13.11 for Observed Total Leaf Values.

On ZAPS II (Table 13.10) the D treatment showed significantly ($p \leq .05$) higher necrosis than the A treatment from early July through mid-August. Plot D was also significantly higher in necrosis than plot B from late June through early August. Finally, the D treatment showed significantly more necrosis than the C treatment in early July. The trend was DCAB until mid-August when it became less consistent. These data are portrayed graphically in Figures 13.37 through 13.39, where plot D is compared to plots A, B, and C.

## DISCUSSION AND SUMMARY

### SULFUR

Sulfur levels in live plant foliage of the seven species of grasses, forbs, and semi-shrubs collected at ZAPS plots during 1976 adequately demonstrate that elevated concentrations are directly related to the duration of fumigation and the $SO_2$ concentrations delivered at the four treatment levels. The foliage of the four species (Agropyron smithii, K. cristata, Achillea millefolium, and Artemisia frigida) which started to grow before fumigations began at ZAPS sites I and II in April of 1976 showed higher sulfur levels than are normally found during the rest of the growing season (i.e., average 1,370 vs. 950 ± 75 ppm). Evidence of residual sulfur concentrations in live tissue resulting from the 1975 fumigation on ZAPS I was demonstrated clearly only in one of the species (A. millefolium) on the B, C, and D treatment plots, while residual sulfur was evident in live foliage of A. frigida from the C and D treatment plots. Neither of the two early season grasses (A. smithii and K. cristata) collected in 1976 demonstrated evidence of elevated residual sulfur concentrations due to 1975

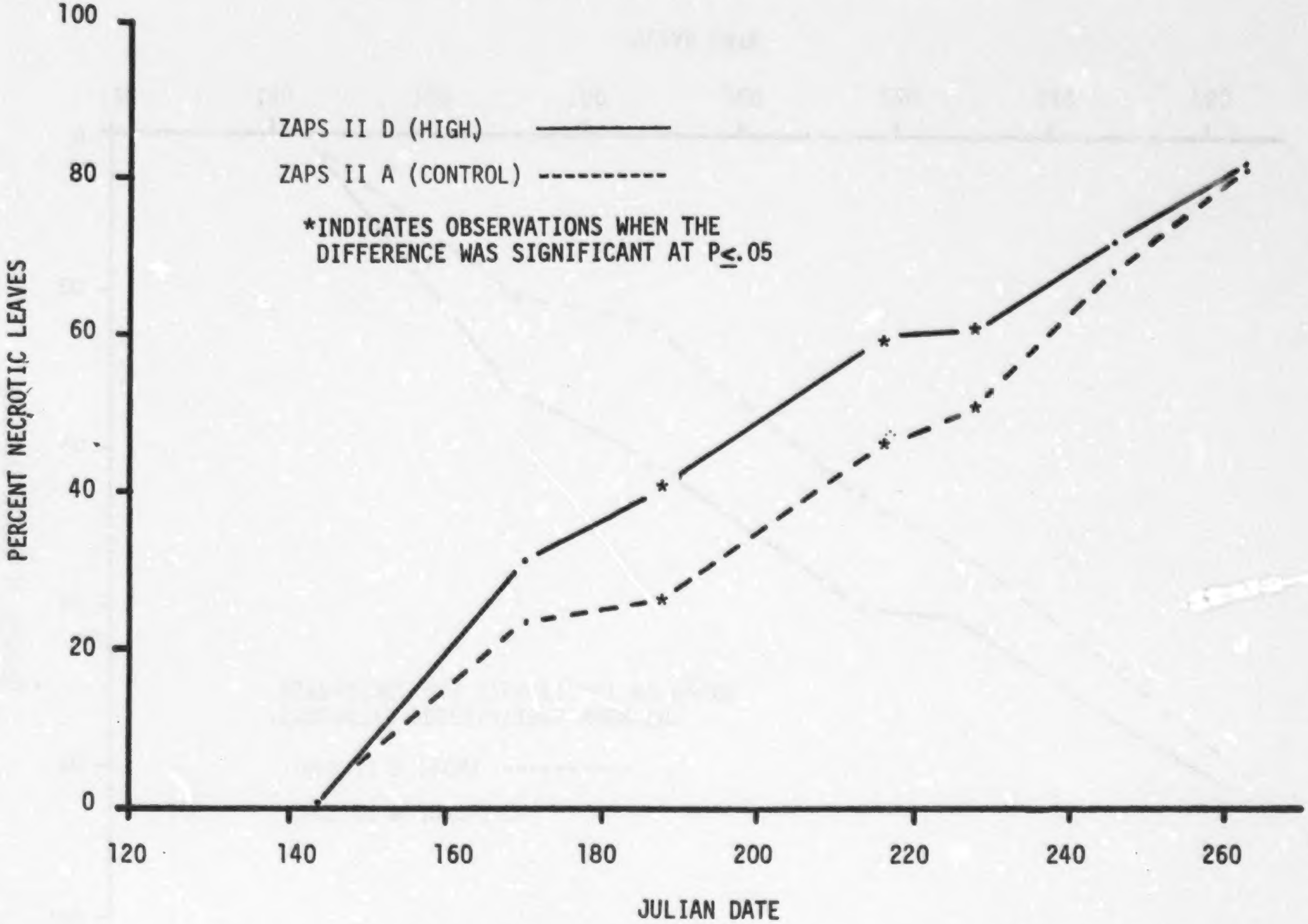

Figure 13.37. Percent necrotic leaves of *Agropyron smithii*.

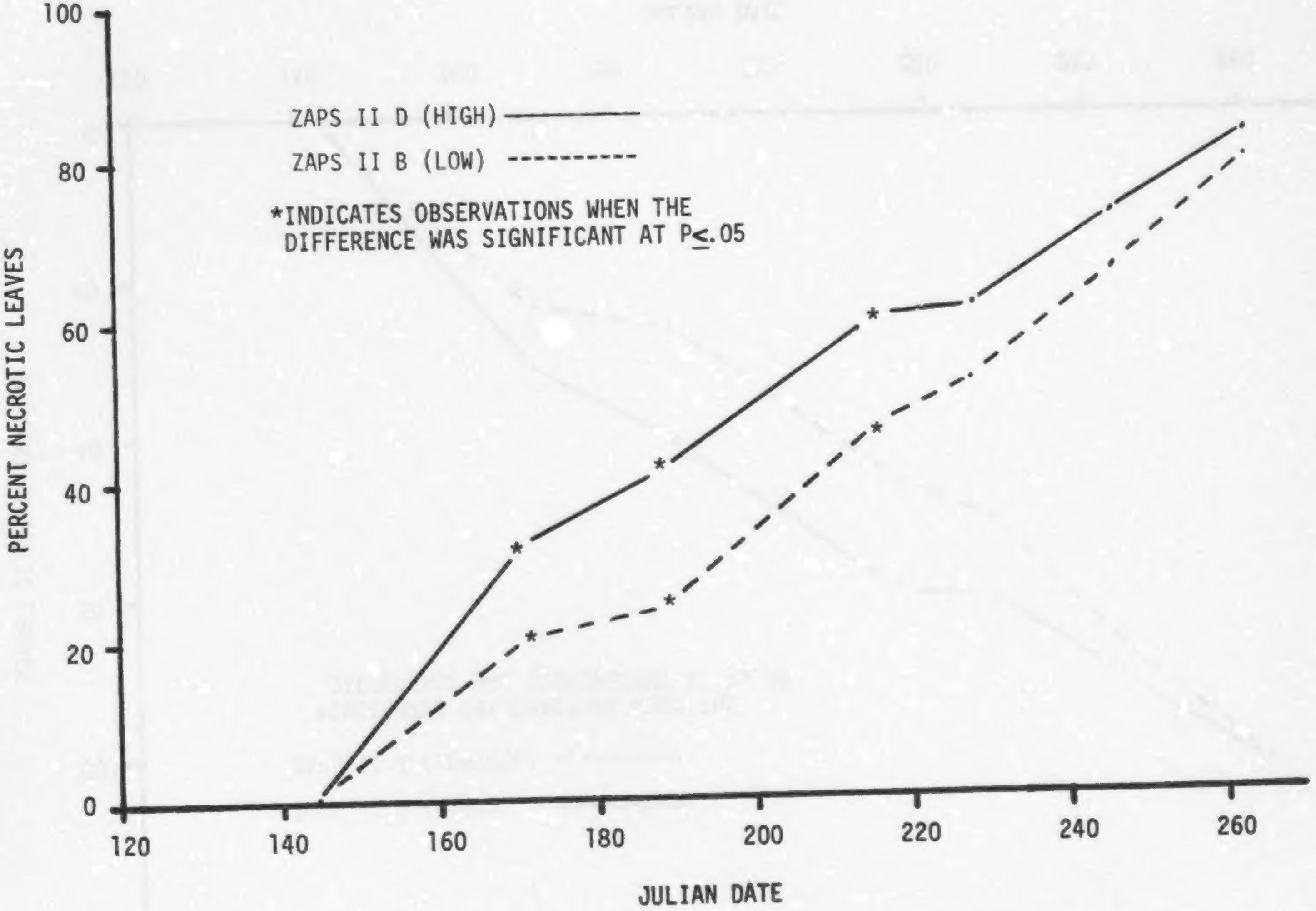

**Figure 13.38.** **Percent necrotic leaves of _Agropyron_ _smithii_.**

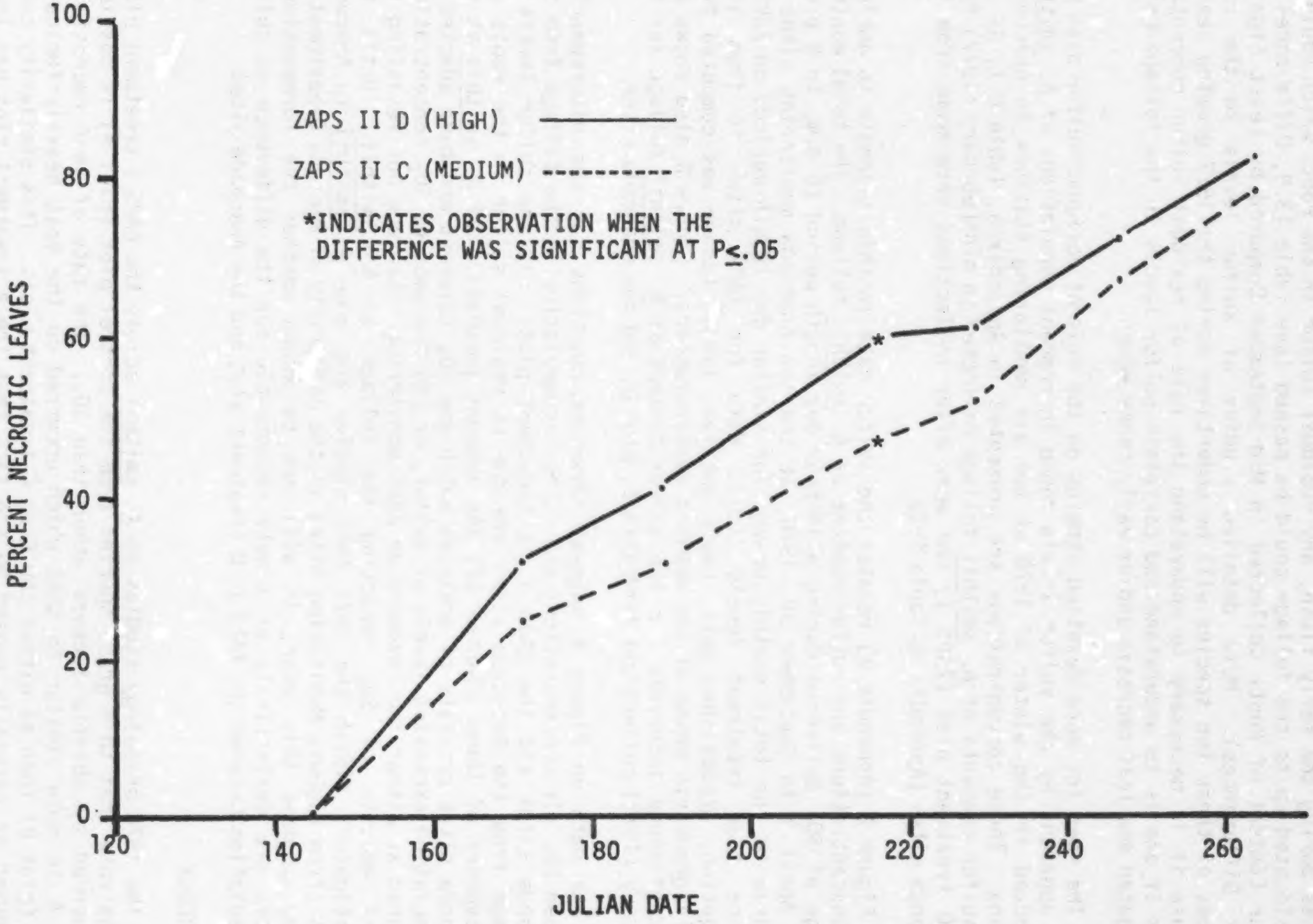

Figure 13.39. Percent necrotic leaves of Agropyron smithii.

fumigation. However, because of the elevated sulfur levels in both of these grasses during the early flush, any residual sulfur in the roots which could be translocated up to the foliage could be masked (see Table 13.8, Differences in Sulfur Content of Roots Collected in Mid-September Compared by Least Significant Differences). More detailed studies of sulfur levels in the root tissues of these two species will be undertaken during the 1977 growing season because it is necessary to understand the role of residual sulfur concentrations if one is to understand and correlate sulfur levels in the foliage being fumigated and leaf necrosis and/or early senescence.

The need for more detailed studies on the role of residual sulfur also was made apparent by the sulfur levels found in remnant populations of *A. smithii* collected in the winter of 1976 at two air monitoring stations in Anaconda, Montana. These concentrations are presented in Appendix B, Table B-1, as are the sulfur contents of *A. smithii* foliage collected in mid-February (1977) from the D treatment plot (ZAPS I) one week after collections were made from the Anaconda sites (Appendix B, Table B-2).

Figure B (Appendix B) relates the sulfur data in this appendix to ambient $SO_2$ concentrations and sulfur content of *A. smithii* foliage. The total monthly dosage of $SO_2$ delivered during a 14-hour day length period (6 a.m. to 8 p.m.) from April 1 to September 30, 1976, at the two Anaconda monitoring sites is compared to the total monthly dosage for similar day length periods on ZAPS I at the four treatment levels (basic data for ZAPS sites is from 1975 fumigation, unpublished data, Lewis and Lee, EPA). Dosage was computed from hourly geometric means of the ambient concentrations. Figure B also shows the 95% confidence intervals for the sulfur content of *A. smithii* foliage for the February (1977) collections from ZAPS I, plot D, and the Anaconda sites.

The data on Figure B suggest three explanations for the discrepancies between $SO_2$ air concentrations and sulfur accumulation in the foliage from the Anaconda sites and the ZAPS I, D treatment plot: (1) The sulfur levels in foliage from the Anaconda sites are due to residual sulfur in the roots and rhizosphere of these plants; (2) the remnant population of *A. smithii* at the Anaconda sites consists of ecotypes which are $SO_2$ tolerant and have adapted to accumulating excessive levels of sulfur, or (3) the ambient $SO_2$ concentrations measured at either the Anaconda or ZAPS monitoring sites are not revealing the actual amounts of $SO_2$ impacting the foliage of *A. smithii*. Until the investigators finish the 1977 ZAPS studies and grow *A. smithii* in Anaconda soils (from Highway Monitoring Site) in the University of Montana Department of Botany gardens this year, it will not be known whether the forementioned factors are collectively or singly responsible for the difference in sulfur accumulation between the ZAPS I, D treatment plot and the Anaconda sites.

## PHENOLOGY

The 1976 phenology studies on *A. smithii* across the ZAPS I treatment plots demonstrate that this grass species from the control plot (plot A) is possibly influenced by abiotic factors other than $SO_2$. The rate of leaf necrosis on plot A is more similar to that which occurred on the most heavily-fumigated plot (plot D) than to either the B or C treatment plots. This similarity could be caused or partially caused by the fact that the A treatment plot has lower soil moisture (1976 soil moisture data from Dodd and Fort Collins, Colorado, study group) in the top 30 cm of soil than the D treatment plot. Differences between soil moisture in the top 30 cm of soil on plots A and D occurred in late June (June 24) and continued on through the remaining growing season. While the overall difference was only 19 percent (i.e., 4.39 g/cm$^2$) from June 24 to September 6 (74 days), this measured reduction of soil moisture on treatment plot A might explain why leaf necrosis at this location is most similar to the leaf necrosis on treatment plot D (see Table 13.10, Phenology of ZAPS I).

The soil moisture profile of all four treatment plots on ZAPS I should be examined thoroughly during the 1977 growing season to ascertain whether there are significant differences between the plots which could be influencing the results of phenology, biomass, and species diversity studies being carried out on ZAPS I.

Phenology studies of A. smithii on ZAPS II (Table 13.11) during the period of mid-June (Julian Day 170) to mid-August (Julian Day 228) demonstrated that $SO_2$ concentrations on that D plot caused significantly more leaf necrosis than that occurring on the other three treatment plots. However, the $SO_2$ concentrations delivered to the B and C treatment plots did not cause a significant difference in leaf necrosis from that occurring on the control plot. This would indicate that the gradient for these concentrations, while causing increases in sulfur accumulation in foliage over baseline, are not adequate to cause differences in visible damage to the foliage of A. smithii.

Phenology studies of A. smithii and one other grass species during the 1977 growing season (possibly K. cristata) will be carried out to compare and verify the results of the 1976 phenology studies.

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## APPENDIX A

### TABLE A-1. ZAPS I - TREATMENT A (CONTROL)
### 1976 COLLECTION

| Collection Date | Julian Date | Sulfur Levels (ppm)<br>*A. smithii* Live | Dead | *K. cristata* Live | Dead | *P. sandbergii* Live | Dead | *A. longiseta* Live | Dead | *A. millefolium* Live | Dead | *A. frigida* Live | Dead |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6 April | 97 | 1300 | 500 | 1675 | 600 | NS | NS | NS | 400 | 800 | 700 | 1100 | 1100 |
| 13 May | 134 | 700 | NS | 700 | NS | 500 | NS | NS | NS | 600 | NS | 1250 | NS |
| 1 June | 153 | 850 | NS | 550 | NS | 550 | NS | NS | NS | 550 | NS | - | NS |
| 18 June | 170 | 1000 | NS | 900 | NS | 550 | NS | NS | NS | 850 | NS | 1550 | NS |
| 4 July | 186 | 700 | 500 | 650 | 550 | NS | 400 | 1000 | NS | 550 | 550 | 1300 | NS |
| 2 August | 215 | 1000 | 900 | 800 | 500 | NS | NS | 750 | 700 | 550 | 650 | 1250 | NS |
| 16 August | 229 | 850 | 750 | 700 | 550 | NS | NS | 950 | 850 | 750 | 600 | 1300 | 1300 |
| 1 September | 245 | 1275 | 1225 | 875 | 750 | NS | NS | 900 | 600 | 900 | 800 | 1550 | 1300 |

NS=not sampled

-=missing sample

## APPENDIX A

### TABLE A-2. ZAPS II - TREATMENT A (CONTROL)
### 1976 COLLECTION

| Collection Date | Julian Date | Sulfur Levels (ppm) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *A. smithii* | | *K. cristata* | | *P. sandbergii* | | *S. viridula* | | *A. millefolium* | | *A. frigida* | |
| | | Live | Dead | Live | Dead | Live | Dead | Live | Dead | Live | Dead | Live | Dead |
| 10 April | 101 | 1716 | 350 | 1575 | 500 | NS | NS | NS | 550 | 1175 | 550 | 1600 | 750 |
| 8 May | 128 | 800 | NS | 650 | NS | 650 | NS | 800 | NS | 850 | NS | 1550 | NS |
| 31 May | 152 | 850 | NS | 750 | NS | 600 | NS | 800 | NS | 700 | NS | 913 | NS |
| 18 June | 170 | 1050 | NS | 900 | NS | 700 | NS | 1100 | NS | 950 | NS | 1350 | NS |
| 6 July | 188 | 1050 | NS | 500 | NS | NS | 450 | 950 | NS | 650 | 650 | 1133 | NS |
| 3 August | 216 | 800 | 800 | 650 | 550 | NS | NS | 1150 | 950 | 600 | 700 | 1250 | NS |
| 15 August | 228 | 950 | 850 | 650 | 700 | NS | NS | 1000 | 750 | 850 | 700 | 1350 | 1050 |
| 1 September | 245 | 800 | 850 | 850 | 650 | NS | NS | 1100 | 850 | 750 | 600 | 1550 | 750 |

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### TABLE A-3. ZAPS I - TREATMENT B (LOW)
### 1976 COLLECTION

| Collection Date | Julian Date | Sulfur Levels (ppm) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *A. smithii* | | *K. cristata* | | *P. sandbergii* | | *A. longiseta* | | *A. millefolium* | | *A. frigida* | |
| | | Live | Dead | Live | Dead | Live | Dead | Live | Dead | Live | Dead | Live | Dead |
| 7 April | 98 | 1400 | 600 | 1575 | 500 | NS | NS | NS | 725 | 1275 | - | 1700 | 1150 |
| 13 May | 134 | 1050 | NS | 900 | NS | 950 | NS | NS | NS | 800 | NS | 1950 | NS |
| 1 June | 153 | 1050 | NS | 900 | NS | 783 | NS | NS | NS | 800 | NS | 1450 | NS |
| 16 June | 168 | 1350 | NS | 1425 | NS | 850 | NS | NS | NS | 1200 | NS | 1850 | NS |
| 5 July | 187 | 1450 | 850 | 1200 | 1050 | NS | 500 | 850 | NS | 700 | 1050 | 1750 | NS |
| 2 August | 215 | 1500 | 1400 | 1850 | 1400 | NS | NS | 1050 | 1050 | 1200 | 1350 | 1933 | NS |
| 16 August | 229 | 1700 | 1650 | 1550 | 1550 | NS | NS | 1200 | 1050 | 1150 | 1400 | 1600 | 1600 |
| 2 September | 246 | 1650 | 2000 | 1800 | 1600 | NS | NS | 1300 | 1150 | 950 | 1225 | 1500 | 1900 |

## APPENDIX A

### TABLE A-4. ZAPS II - TREATMENT B (LOW)
### 1976 COLLECTION

| Collection Date | Julian Date | Sulfur Levels (ppm) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *A. smithii* | | *K. cristata* | | *P. sandbergii* | | *S. viridula* | | *A. millefolium* | | *A. frigida* | |
| | | Live | Dead | Live | Dead | Live | Dead | Live | Dead | Live | Dead | Live | Dead |
| 10 April | 101 | 1250 | 600 | 1050 | 550 | NS | NS | NS | - | 850 | 700 | 2125 | 1050 |
| 11 May | 132 | 1100 | NS | 800 | NS | 975 | NS | 1275 | NS | 1050 | NS | 1350 | NS |
| 1 June | 153 | 1300 | NS | 1100 | NS | 800 | NS | 950 | NS | 1050 | NS | 1050 | NS |
| 21 June | 173 | 1450 | NS | 850 | NS | 950 | NS | 1350 | NS | 1800 | NS | 1900 | NS |
| 7 July | 189 | 1600 | NS | 1300 | NS | NS | 850 | 1600 | NS | 1100 | 1400 | 1950 | NS |
| 3 August | 216 | 1550 | 1900 | 1550 | 1350 | NS | NS | 1300 | 1200 | 1300 | 1550 | 1750 | NS |
| 15 August | 228 | 1250 | 1500 | 1050 | 1250 | NS | NS | 1100 | 1350 | 775 | 1300 | 1550 | 1350 |
| 2 September | 246 | 1450 | 1950 | 1350 | 1300 | NS | NS | 1600 | 1500 | 1350 | 1350 | 1800 | 1500 |

## APPENDIX A

### TABLE A-5. ZAPS I - TREATMENT C (MEDIUM)
### 1976 COLLECTION

| Collection Date | Julian Date | *A. smithii* |  | *K. cristata* |  | *P. sandbergii* |  | *A. longiseta* |  | *A. millefolium* |  | *A. frigida* |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  |  | Sulfur Levels (ppm) |  |  |  |  |  |  |  |  |  |  |  |
|  |  | Live | Dead | Live | Dead | Live | Dead | Live | Dead | Live | Dead | Live | Dead |
| 7 April | 98 | 1300 | 800 | 1200 | 850 | NS | NS | NS | 750 | 900 | 1050 | 2700 | 1100 |
| 13 May | 134 | 1350 | NS | 1200 | NS | 1150 | NS | NS | NS | 1150 | NS | 2050 | NS |
| 1 June | 153 | 1500 | NS | 1600 | NS | 1450 | NS | NS | NS | 1450 | NS | 2050 | NS |
| 16 June | 168 | 2050 | NS | 2250 | NS | 988 | NS | NS | NS | 1650 | NS | - | NS |
| 5 July | 187 | 1750 | 1200 | 2100 | 1250 | NS | 900 | 1450 | NS | 2000 | 2700 | 3100 | NS |
| 2 August | 215 | 2550 | 2750 | 2350 | 2200 | NS | NS | 1250 | 1400 | 2200 | 2850 | 2700 | NS |
| 16 August | 229 | 2550 | 2950 | 2400 | 2000 | NS | NS | 1750 | 1700 | 1850 | 2150 | 2350 | 2325 |
| 2 September | 246 | 2225 | 2800 | 2600 | 1800 | NS | NS | 1275 | 1500 | 2150 | 2350 | 3150 | 2100 |

# APPENDIX A

## TABLE A-6. ZAPS II - TREATMENT C (MEDIUM)
## 1976 COLLECTION

| Collection Date | Julian Date | Sulfur Levels (ppm) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *A. smithii* | | *K. cristata* | | *P. sandbergii* | | *S. viridula* | | *A. millefolium* | | *A. frigida* | |
| | | Live | Dead | Live | Dead | Live | Dead | Live | Dead | Live | Dead | Live | Dead |
| 9 April | 100 | 1100 | 400 | 1675 | 550 | NS | NS | NS | 450 | 550 | 500 | 1250 | 800 |
| 11 May | 132 | 1150 | NS | 1550 | NS | 1450 | NS | 2500 | NS | 1400 | NS | 1650 | NS |
| 31 May | 152 | 1700 | NS | 1300 | NS | 1250 | NS | 1600 | NS | 1200 | NS | 1750 | NS |
| 21 June | 173 | 2200 | NS | 2250 | NS | 1250 | NS | 2250 | NS | 2550 | NS | - | NS |
| 7 July | 189 | 2150 | NS | 2450 | NS | NS | 1100 | 2600 | NS | 2200 | 2450 | 2325 | NS |
| 3 August | 216 | 2616 | 2850 | 2650 | 2250 | NS | NS | 2100 | 2500 | 2350 | 3750 | 3150 | NS |
| 15 August | 228 | 2200 | 3400 | 2250 | 1950 | NS | NS | 2250 | 2250 | 2150 | 2800 | 3050 | 3350 |
| 2 September | 246 | 2450 | 3200 | 2550 | 2050 | NS | NS | 2950 | 2800 | 1800 | 2250 | 3950 | 2800 |

## APPENDIX A

### TABLE A-7. ZAPS I - TREATMENT D (HIGH)
### 1976 COLLECTION

| Collection Date | Julian Date | *A. smithii* Live | *A. smithii* Dead | *K. cristata* Live | *K. cristata* Dead | *P. sandbergii* Live | *P. sandbergii* Dead | *A. longiseta* Live | *A. longiseta* Dead | *A. millefolium* Live | *A. millefolium* Dead | *A. frigida* Live | *A. frigida* Dead |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Sulfur Levels (ppm) | | | | | | | | | | | |
| 8 April | 99 | 1500 | 1000 | 1200 | 1050 | NS | NS | NS | 800 | 1350 | 1600 | 2650 | 2725 |
| 13 May | 134 | 1750 | NS | 1650 | NS | 1550 | NS | NS | NS | 1300 | NS | 3300 | NS |
| 1 June | 153 | 1950 | NS | 2017 | NS | 1500 | NS | NS | NS | 2750 | NS | 3400 | NS |
| 16 June | 168 | 2400 | NS | 3350 | NS | 1900 | NS | NS | NS | 3600 | NS | - | NS |
| 4 July | 186 | 2600 | 1519 | 3850 | 2100 | NS | 2350 | 1850 | NS | 3725 | 5750 | 5150 | NS |
| 2 August | 215 | 3175 | 4725 | 4275 | 2850 | NS | NS | 2100 | 1700 | 3900 | 5250 | 5450 | NS |
| 16 August | 229 | 3450 | 3200 | 3400 | 2350 | NS | NS | 2350 | 1800 | 4400 | 6367 | 4250 | 4200 |
| 2 September | 246 | 3800 | 4100 | 3400 | 2650 | NS | NS | 2400 | 2100 | 5325 | 4750 | 5550 | 3850 |

## APPENDIX A

### TABLE A-8. ZAPS II - TREATMENT D (HIGH)
### 1976 COLLECTION

| Collection Date | Julian Date | Sulfur Levels (ppm)<br>A. smithii Live | Dead | K. cristata Live | Dead | P. sandbergii Live | Dead | S. viridula Live | Dead | A. millefolium Live | Dead | A. frigida Live | Dead |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 9 April | 100 | 1400 | 450 | 1625 | 600 | NS | NS | NS | 500 | 500 | 550 | 1450 | 850 |
| 13 May | 134 | 1850 | NS | 1300 | NS | 1400 | NS | 3250 | NS | 2600 | NS | 2700 | NS |
| 31 May | 152 | 3250 | NS | 1550 | NS | 1900 | NS | 2150 | NS | 2800 | NS | 3550 | NS |
| 21 June | 173 | 4350 | NS | 3750 | NS | 2400 | NS | 4650 | NS | 4983 | NS | - | NS |
| 7 July | 189 | 4200 | NS | 4183 | NS | NS | 1800 | 4850 | NS | 3875 | 4750 | 4850 | NS |
| 3 August | 216 | 4150 | 5675 | 3550 | 3250 | NS | NS | 3600 | 3000 | 4100 | 5400 | 5150 | NS |
| 15 August | 228 | 3100 | 3950 | 3550 | 3800 | NS | NS | 4150 | 3800 | 4950 | 5500 | 5450 | 5925 |
| 2 September | 246 | 3950 | 4450 | 3950 | 2900 | NS | NS | 4250 | 4000 | 6000 | 4550 | 5100 | 4350 |

## APPENDIX B

TABLE B-1. COLLECTION OF *Agropyron smithii* AT TWO ANACONDA MONITORING SITES, 1976.

| Site | | Sulfur Concentrations (ppm) |
|---|---|---|
| Highway Junction Monitoring Site | 3500 | Arithmetic Average 0.02 |
| | 3550 | Maximum 1 hr. Ave. 1.52 |
| | 4025 | Maximum 3 hr. Ave. 1.25 |
| | 3750 | Maximum 24 hr. Ave. 0.48 |
| | 3700 | |
| | 3000 | |
| | Average 3587 | |
| | | Number of Monitoring Days 329 |
| | | Total 1 hr. Readings Taken 7665 |
| Mill Creek Monitoring Site | 2400 | Arithmetic Average 0.014 |
| | 2150 | Maximum 1 hr. Ave. 0.79 |
| | 1950 | Maximum 3 hr. Ave. 0.48 |
| | 1950 | Maximum 24 hr. Ave. 0.13 |
| | 2050 | |
| | 1950 | |
| | 2650 | |
| | 2950 | Number of Monitoring Days 317 |
| | 3000 | |
| | 1950 | Total 1 hr. Readings Taken 7202 |
| | Average 2300 | |

TABLE B-2. PPM SULFUR IN *Agropyron smithii* FROM ZAPS I, TREATMENT D collected February 16, 1977.

| |
|---|
| 1450 |
| 1550 |
| 1300 |
| 1350 |
| 1100 |
| 1500 |
| 1250 |
| 1200 |
| 1450 |
| 1500 |

$\bar{x} = 1365$ $s_x = 149$ $s_{\bar{x}} = 47$ $n = 10$

# APPENDIX B

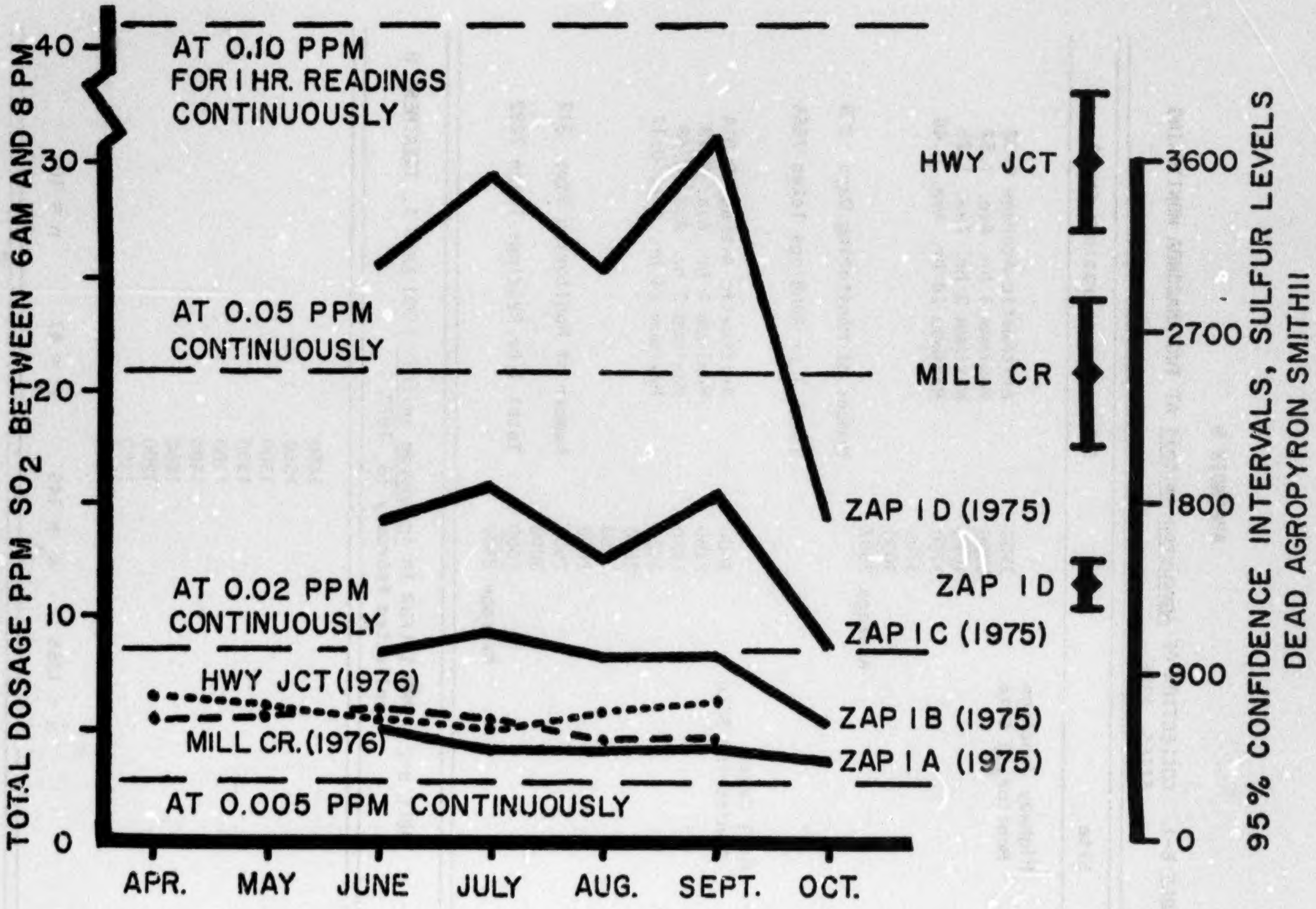

Figure B. Dosage and accumulation of sulfur in *Agropyron smithii*.

## SECTION 14

# INVESTIGATION OF THE IMPACT OF COAL-FIRED POWER PLANT EMISSIONS UPON INSECTS: ENTOMOLOGICAL STUDIES AT THE ZONAL AIR POLLUTION SYSTEM

by

J.J. Bromenshenk

## INTRODUCTION

This portion of the report concentrates on the entomological investigations at the EPA sulfur dioxide fumigation plots (Zonal Air Pollution System-ZAPS) at Taylor Creek, near Fort Howes, Montana, for the periods of June 15 through October 20, 1975, and May 1 through November 15, 1976. During 1975, attention was given primarily to collecting baseline data in the vicinity of Colstrip, Montana, since baseline conditions would no longer prevail there once the Colstrip power plants began operation. The first plant began to burn coal in early September 1975. With baseline data banks established (see sections on animal and vegetation studies in the vicinity of Colstrip, Montana, by Gordon, Tourangeau, and Rice and the entomological investigations by Bromenshenk), attention was redirected to the ZAPS.

Investigations were initiated in the late summer of 1975 and efforts were intensified in 1976. The reason for the intensification of the work at ZAPS was twofold: (1) The baseline data collections were completed and the study had entered a monitoring phase; and (2) we were convinced that the Zonal Air Pollution System could be useful in testing the effects of sulfur dioxide ($SO_2$) upon plant and animal (arthropod) species or populations. This belief was based on first season evaluations of field experiments, the system design, and testing of the gas delivery. These indicated that measurable changes in response to the $SO_2$ had occurred (unpublished reports to EPA).

Experiments were conducted to study the behavioral and physiological responses of honeybees to in situ $SO_2$ fumigation in 1975, and in 1976, studies were initiated of the inter- and intra-plot distributions of other insect species: Ground-dwelling beetles, airborne or flying insects, and native pollinators.

As noted previously, this research is one aspect of the Colstrip project to develop methods to predict the bioenvironmental impacts of emissions from coal-fired power plants before any damage actually occurs. The most prevalent toxic, gaseous materials released by the combustion of coal are sulfur oxides (DHES, 1975; WES, 1973). Because of their abundance, consequences to human and vegetative health, and deteriorative effects, oxides of sulfur are significant air pollutants (Smith, 1975). They may impact ecosystems as gaseous oxides, as acids in precipitation (Likens, 1974), or as synergists with other pollutants.

Known interactions of insects and sulfur compounds include: (1) The use of $SO_2$ as a fumigant against stored product and household pests (Kanaga, 1956; Swisher, 1944; Negherbon, 1966); (2) changes in the population dynamics of forest insects induced by $SO_2$ air pollution stress (Linzon, 1966; Bösener, 1969; Donaubauer, 1968; Scheffer and Hedgcock, 1955; Kudela, 1962; Sierpinski, 1966, 1967; Templin, 1962); (3) decreases in populations of social bees and parasitic wasps and increases in aphids linked to $SO_2$ stress from a 615 MW coal-fired power plant in Pennsylvania (Hillmann and Benton, 1972); (4) decreases in populations of ground-dwelling beetles, mostly Carabids, near a Kraft mill in Canada (Frietag et al., 1973), and (5) reductions in brood rearing, reduced pollen collection, depressed flight activity, and uncertain mortality effects produced by fumigation for 9- and 14- week periods using 0 to 5 ppm $SO_2$ on 20 honeybee colonies (Hillmann and Benton, 1972). They noted that reduced flight activity contributed to decreased honey production.

These literature reports suggest: (1) Trees or plants which are physiologically weakened by air pollutants such as $SO_2$ become more susceptible to attack by those insects which seek out weak plants for feeding and reproduction; (2) host-predator and host-parasite imbalances may occur, probably as a result of a concentration of toxic substances in food-chains, and (3) pollinators, namely honeybees and social bees, demonstrate a variety of physiological and behavioral responses to $SO_2$. In his review of air pollution and apiculture (1972), Debackere discussed the chemical reactions in the atmosphere which convert $SO_2$ to sulfurous acid ($H_2SO_3$) and sulfuric acid ($H_2SO_4$). He reported that it is well known that these sulfur compounds have an irritating effect on the gastrointestinal tract of mammals. Although almost nothing is known about the effects on bees as indicated by literature reports, it is assumed that these acids have a deleterious effect on the intestinal tract of bees. The ground beetle studies of Frietag et al. (1973) demonstrated significant decreases in the numbers of Carabids associated with increased fall-out of $SO_4^{2-}$ in the form of sodium sulfate ($Na_2SO_4$); these beetles were all predatory species. Kulman (1974) reported that Carabids are sensitive to most insecticides and pollutants and that it appeared that seed-eating beetles contain fewer ppm of insecticides than predatory beetles. A bibliography of literature pertaining to air pollution and insects is included as Appendix A in section 5; a more in-depth literature review appears in the section on the entomological studies near Colstrip.

In the introduction to a problem analysis of air pollution in urban areas which divides air pollution influences on temperate forest systems into three classes based on pollution load, Smith (1975) presented the known responses of trees to these loads and indicated associated ecosystem impacts. This breakdown provided a generalized conceptual model for other ecosystems; his model is presented in Table 14.1.

TABLE 14.1. INFLUENCE OF AIR POLLUTION ON TEMPERATE FOREST ECOSYSTEMS[1]

| Designation | Air Pollution Load | Response of Trees | Impact on Ecosystems |
|---|---|---|---|
| Class I | Low | 1. Act as a sink for contaminants | 1. Pollutants shifted from atmospheric to organic or available nutrient compartment |
| | | 2. No or minimal physiological alteration | 2. Undetectable influence or fertilizing effect |
| Class II | Intermediate | 1. Reduced growth<br>a. detruded nutrient availability<br>1) depressed litter decomposition<br>2) acid rain leaching<br>b. suppressed photosynthesis enhanced respiration | 1. Reduced productivity, lessened biomass |
| | | 2. Reduced reproduction<br>a. pollinator interference<br>b. abnormal pollen, flower, seed, or seedling development | 2. Alteration of species composition |
| | | 3. Increased morbidity<br>a. predisposition to entomological or microbial stress<br>b. direct disease induction | 3. Increased insect outbreaks, microbial epidemics |
| Class III | High | 1. Acute morbidity | 1. Simplification: increased erodibility, nutrient attrition, altered microclimate and hydrology |
| | | 2. Mortality | 2. Reduced stability |

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[1] From Smith, 1975

Because the Colorado investigative team (CNREL) conducted tests to determine the effects of sulfur dioxide fumigation on the density, biomass, and trophic composition of above-ground and below-ground arthropods, this research concentrated on a few insect species which, according to literature reports, were sensitive or susceptible to perturbations, easy to monitor and measure, and reliable predictors of bionomic and economic impacts. In addition, these species could be related to the studies in the Colstrip area and to the needs of the overall project. Honeybees were used as detectors and accumulators of air pollutants at apiary locations throughout southeastern Montana. These bees have an estimated economic value of $6 to $33 million as pollinators and $300 to $725,000 as honey producers annually (Montana Agricultural Reports, 1977; McGregor, 1976), have bionomic value as pollinators of rangelands and croplands, and have proved to be extremely sensitive and useful indicators of a variety of atmospheric pollutants (see section on Colstrip vicinity studies). Smith (1975) listed pollinator interference and reduced reproduction, which alters plant species composition, as an important effect of intermediate air pollution stress on ecosystems. Therefore, high priority was placed on studying the effects of $SO_2$ fumigation on honeybees.

Data collected by the Colorado team in 1975 (unpublished reports) indicated that during the first year of fumigation no order of arthropods showed any treatment response except the Lepidopterans, which are plant tissue and nectar feeders. They were lowest in the highest fumigation (10 pphm) plot; however, low population numbers made the results inconclusive. The Colorado investigators noted that scavengers, represented primarily by Collembolan species, decreased in numbers in the high plot and that numbers of Carabid beetles also may have changed in response to the highest fumigation levels. Again, the population numbers were too low to make any definitive statement. Finally, more grasshopper eggs were found on the control plot, an area that from observations had less cover and somewhat different soil characteristics than the other plots. The Colorado group added investigations of grasshoppers in 1976; the investigators of this research added studies of ground-dwelling beetles (predators and scavengers) and of native nectar feeders (Lepidopterans, and pollinating Dipterans and Hymenopterans, namely native bees).

The main objectives of the entomological studies at ZAPS were: (1) To establish honeybee colonies for behavioral and physiological experimentation; (2) to conduct preliminary tests of experimental procedures and equipment to maximize the potential usefulness of the ZAPS system; (3) to evaluate the initial responses of honeybees and other insects to *in situ* $SO_2$ fumigation so that further research directions (e.g., process and mechanism studies) could be determined, and (4) to evaluate intra- and inter-plot distribution of the selected species of insects via grid trapping and gradient analysis.

We were interested in characterizing species of insects and temporal (season) differences in susceptibility to $SO_2$ stress in the cool season grassland. The hypotheses were that: (1) Insects of the higher trophic levels such as predators, scavengers, and pollinators would be more susceptible to damage than foliage feeders; (2) the effects of $SO_2$ fumigation would be reflected by behavioral and physiological responses of the target organism; (3) these behavioral and physiological responses could be observed and measured (e.g., distributions, immigration, emigration, mortality, shortened life span, changes in fecundity and fertility, etc.); and (4) that long-term chronic exposure to $SO_2$ would be reflected by increased susceptibility, increased resistance, elimination, or no discernible effects.

## MATERIALS AND METHODS

To reduce physiological and/or behavioral responses due to genetic variability, in May 1975, the USDA Bee Stock Center (Baton Rouge, Louisiana) provided eight dihybrid sister queens which had been artificially inseminated with sperm from drones of a single inbred line (see Appendix B for a description). These queens were introduced into eight colonies and used to convert the colonies to the "same" genetic composition. Four colonies were moved to the Taylor Creek site; the other four were left at Broadus, Montana, to serve as controls and to provide replacements. By late August 1975, all of the original queens had died or disappeared, although daughter queens continued three colonies at Taylor Creek and one colony at Broadus. Dr. Harbo of the Bee Stock Center believed that the problem of the supercedure of the queens was due to inadequate sperm reserves and/or to injury caused during artificial insemination. The Bee Stock Center provided replacements in May 1976. This line of bees has continued in use because they have proved to be docile in temperament and as such caused a minimum of disturbance to investigators at the ZAPS sites. Due to the lag time of reestablishing colonies from introduced queens, four colonies of "local" bees, provided by a commercial keeper, were utilized at ZAPS I in 1976.

The glass observation hives described in the Second Interim Report were not colonized in 1975 because of the supercedure problem.

During the 1975 and 1976 growing seasons, a beehive was set 70 m above (north) each of the ZAPS I treatment plots. The hives were not set on the plots because of potential encounters between bees and the crews conducting the biological sampling. It was requested that the gas delivery line be extended from each of the treatment plots to the respective hive, but the EPA design engineers concluded that this might alter the delivery flow patterns of the entire system. Thus, we could perform only those experiments which involved the foraging of the bees on the plots, but could not control the exposure, if any, of the bees to the $SO_2$.

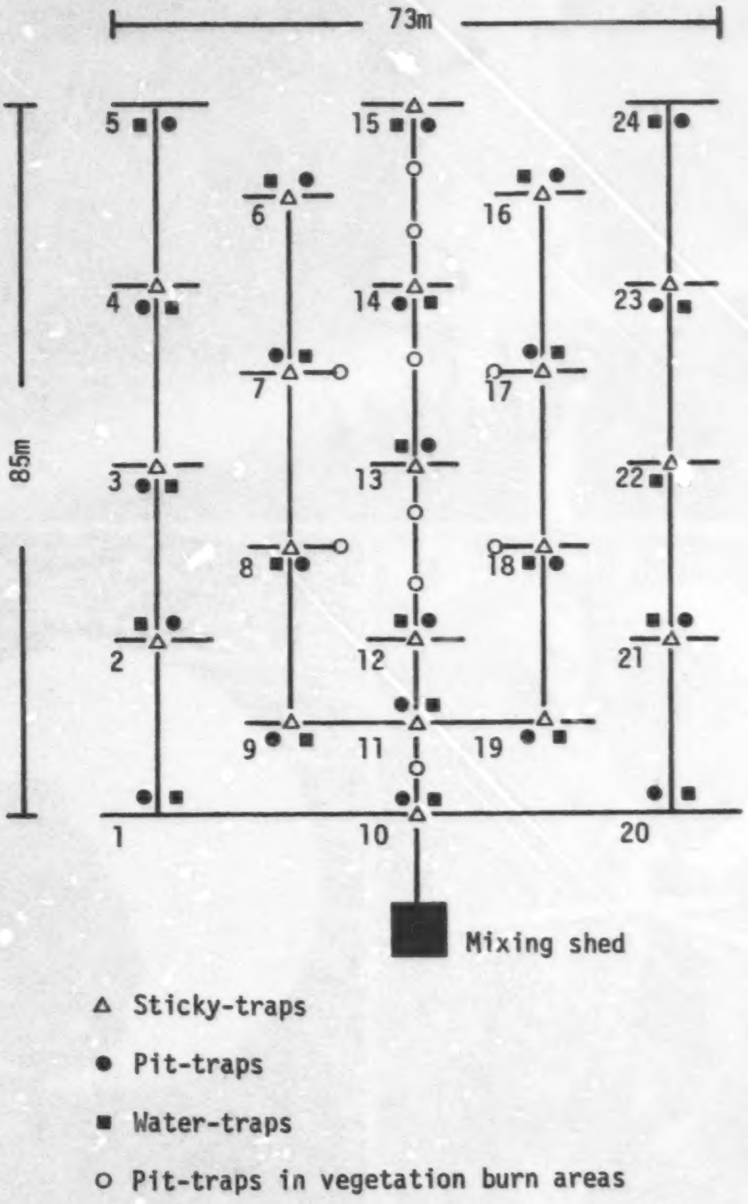
Because of uncertainty about the dosages of $SO_2$ to which the honeybees were being exposed and because we were interested in the effects of $SO_2$ on native pollinators, a trapping series was begun in 1976 designed to capture samples of several species of solitary and social bees (Hymenoptera) and flies (Dipterans) on each of the treatment plots. Drop-traps were based on a design by Olsen, Elliot, and Associates (1976) which was economical, practical, and well-suited for this purpose. They reported that these traps appear to act as artificial flowers. Each trap was composed of a 14-ounce disposable white, plastic beverage container. A white, plastic, conical disposable coffee cup from which the bottom had been removed was inserted into the mouth of each beverage cup. The coffee cup was held in place by a piece of strapping tape. Thus, each trap was a simple funnel-trap. Each cup was set into the ground so that the upper two-thirds extended above the surface of the ground. Approximately one inch of water was placed in the bottom of each beverage cup to drown any captured insects. The cups were inspected and insects removed at approximately ten-day intervals. Twenty of these traps were installed on each of the eight treatment plots for a total of 160 traps (Figure 14.1).

The data from the CNREL group suggested that ground-dwelling beetles were affected by the $SO_2$; literature reports suggested the same. Therefore, a network (Figure 14.1) of pit-fall traps was set on each treatment plot. The traps consisted of the nested cup design utilized to obtain pollinators. Olsen, Elliott, and Associates (1976) found that sinking these cups deeper into the ground decreased their attractiveness to pollinators but greatly increased the catch of beetles (personal communication, D. Schmidt, entomologist). In each treatment plot, 24 of these traps were arranged into a grid pattern and sunk into the ground so that the mouth was level with the surface of the soil. In addition, ten extra cups were placed on ZAPS II below the $SO_2$ gas delivery line of the high (10 pphm) concentration fumigation plot in areas of severe vegetation burn, and ten extra cups were placed on the control plot in the same relative position as the cups on the high concentration plot. Thus, each network consisted of 212 collection points (Figure 14.1) utilized for approximately ten-day intervals during the first half of each month from May through September. An inch of water in the bottom of each trap drowned the beetles and prevented the predacious species from devouring each other and the other captured insects.

The number of beetles obtained in this manner declined rapidly after mid-June and introduced a problem of random-baiting effect because a trap containing a few beetles became much more attractive than one without any beetles. To minimize this effect and to increase trapping success, all traps were baited with decayed meat. This appeared to resolve the immediate problem and greatly improved the trapping yield of Scarabaeids and Silphids (Figure 14.2).

We recognize the limitations of pit-fall trapping and of the possible sources of error resultant from baiting. However, we agree with the statement of Kulman in his 1974 review of the ecology of the North American Carabidae in which he remarked that the pit-fall method persists in studies of ground-dwelling beetles because "...other sampling methods (mark capture, total population over unit area, etc.) also have problems and are more difficult to use."

Finally, to study the distribution of pollinators and of other airborne or flying insects over the plots, which the D-Vac sampling systems utilized by the Colorado entomological investigative team did not adequately sample, a series of cylindrical, sticky traps were employed. Each trap consisted of a 14-ounce disposable plastic beverage cup, coated with Tack Trap®, and taped above each junction of the delivery pipeline (Figure 14.2). The sticky traps were replaced every two weeks. Since color may affect capture, both white and yellow cups were used. Reportedly, native pollinators are attracted to white (Olson, Elliott, and Associates, 1976), while aphids


73m
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1
10
20
Mixing shed
△ Sticky-traps
● Pit-traps
■ Water-traps
○ Pit-traps in vegetation burn areas

Figure 14.1. Distribution of insect traps on ZAPS plots - Summer 1976.

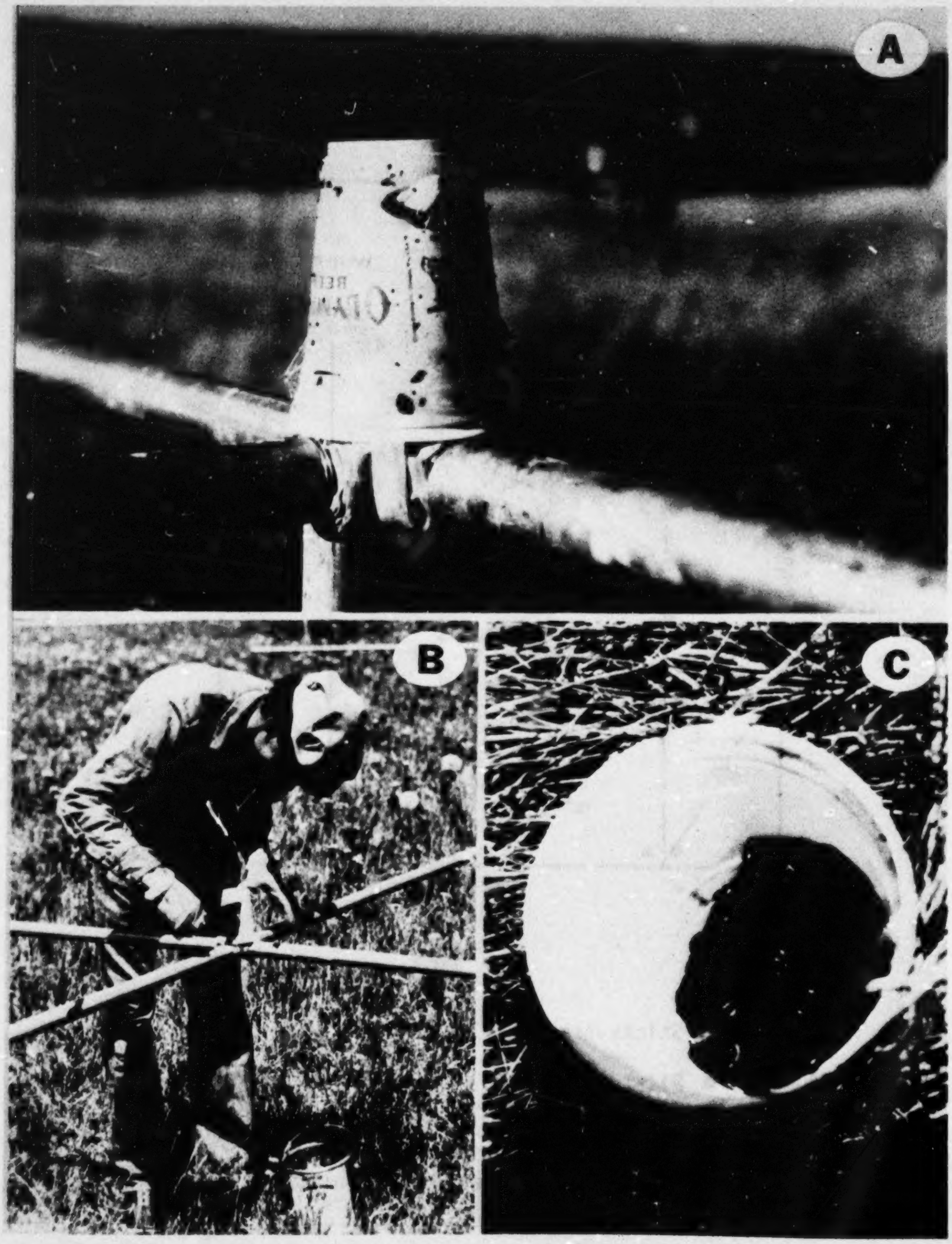

Figure 14.2. A. Sticky-trap taped to $SO_2$ delivery line. B. Coating sticky-trap cup. C. Pit-fall trap and captured beetles. Cup was raised slightly for photographic purposes.

are attacted to yellow (Broadbent, 1948). Hillman reported increased aphid numbers in areas subjected to elevated levels of sulfur from a Pennsylvania coal-fired power plant.

Presumably, color has the greatest effect at low wind speeds (Southwood, 1975). Taylor (1962) demonstrated that a white sticky trap caught insects almost as if they were inert particles, and he constructed tables relating the efficiency of cylindrical traps for different insects at different wind speeds. The relation of catch to size of sticky traps was investigated by Heathcote (1957) and by Staples and Allington (1959). They found that catch usually increased with trap size, but this increase was not proportional to trap size; the smallest trap caught the largest number of insects per unit area. Based on these findings, we decided to test the effects of color. During each six-week trap period, ten white and ten yellow stock-cups were used on each treatment plot for two weeks, then 20 yellow cups for two weeks, followed by 20 white cups for two weeks, at the end of which time the cycle was repeated. When two colors were used, the colors were alternated in each row of traps.

Captured insects were frozen until they could be identified and counted. The number of insects captured per cup per trap day was calculated for purpose of analyses. Destroyed (hail, mice, wind) or upset cups were not included in the analyses. All traps (sticky, drop, and pit-fall) were laid out in a grid system based on the pattern established by the gas delivery line. The pipe junctions were used as sampling points. Each cross-shaped junction visually marked out four quadrants. Quadrants falling outside the north and south perimeter of the delivery system were considered to fall outside of the treatment plot. The traps were arranged in a systematic manner so that each junction and quadrant was utilized, each quadrant being replicated an equal number of times within a plot. Trap locations from treatment plot to plot were identical. Within each "junction quadrant" the pit-fall or drop trap location was determined by measuring 125 cm from the horizontal gas pipeline diagonally to the ground at the first orifice away from the junction. This placed each trap just over one meter from the line. A systematic grid trapping network was used to study dispersion patterns as well as changes in population size.

During 1975, dead-bee traps, pollen traps, magnetic labels, and feeding stations were used in tests on honeybee responses to the fumigations. The dead-bee traps consisted of plastic traps below the entrance of each hive, enclosed by a 2-cm mesh to the inch screen. Living bees could easily fly or crawl through the screen, but housecleaning bees could not carry the dead bees out. Dead bees in the traps were counted daily during those periods when investigators were at the ZAPS plots. Numbers of bees that died away from the hive were not included in the analyses.

The original design for a pollen trap, which is part of the dead bee trap (EPA, 1976), was based on a design by Hillmann (1972). However, this design restricted the entrance to the hive, and the bees piled up at the entrance on days when they were actively foraging. Therefore, a pollen trap based on the design by Henderson (1962) was used. This consisted of a deep honey super (box) with a 2-cm mesh screen over its top, which then became the new entrance. The pollen trap was set under the rest of the hive structure. Bees entered via a normal entrance, then crawled through the screen which formed a false bottom in the hive. The screen brushed pollen off the bees' legs. A pull-out drawer lined with plastic sheeting allowed for the removal of the pollen.

We used a ferrous metal tag capture-recapture technique (Gary, 1971) when appropriate for distribution, movement, and flight tests. Coded metal tags were glued to the abdomen of bees captured in the field. These tags were retrieved at the entrance to the hives by a strip of ceramic magnets. The magnets either pulled the tags off the bees as they entered, or else both tags and bees stuck to the magnet but the bees were able to wiggle free within a short period leaving the tag behind. The ferrous metal technique demonstrated a 78 percent retrieval success, which is consistent with the success ratio reported by Gary (1971). Gary discussed reasons for less than 100 percent retrieval in his paper.

For some experiments, bees were marked with dots of enamel paint (color-coded to each hive). Direct counts of bee visitations and foraging on each plot or at feeding stations were made by walking transects across the plots.

Behavioral tests using feeding stations (glass-watch glasses on which jars were inverted and filled with a one molar sucrose solution as described by von Frisch, 1967) were conducted in late August and early September of 1975. At that time very few flowering plants were in blossom, and honeybees were easily and rapidly trained to feeding stations. During this same 1976 period, honeybees were attracted to the water-containing drop traps and captured, although at other times honeybees were seen only infrequently in or around these traps. Apparently, the shortage of water in nearby reservoirs forced the bees to the traps.

Chi square analyses of variance were used to test whether number of honeybees or other insects on the fumigation plots fit an equal distribution ratio or whether numbers fit some other ratio such as dependency on fumigation level. Regression and co-variance analyses were applied where appropriate, but for the 1976 period the tests relied on the theoretical levels of mean $SO_2$ concentration because the actual data were not available. These analyses are useful approximations but the actual data are needed before more intensive and accurate mathematical methods can be applied, such as within plot distribution.

## RESULTS

Chi square analyses of variance were used to test whether numbers of honeybees on the fumigation plots fit an equal distribution ratio or whether numbers of bees were dependent on the concentration of $SO_2$. The following observations were made at the ZAPS sites during 1975:

(1) Honeybees fed at all feeding stations, even when a feeding station was placed directly under a point source emitting concentrated $SO_2$.

(2) The number of honeybees at each feeding station increased from the time each experiment was initiated, indicating either recruitment or discovery by other bees.

(3) The number of bees at a feeder was greatest just after the sucrose solution was depleted and then decreased rapidly if the feeder was not refilled.

(4) The bees generally were not successful in locating a feeding station that was hidden from sight, even when fragrance (an essential oil such as peppermint) was added.

(5) Marked and tagged bees from each of the colonies (located 75 m north of the fumigation areas) were observed on the plots; no preferential response to $SO_2$ as indicated by the number of bees visiting the feeding stations or different plots could be discerned (Table 14.2).

TABLE 14.2. VISITS BY ADULT WORKER HONEYBEES TO FEEDING DISHES (ONE MOLAR SUCROSE), ZAPS I, AUGUST-SEPTEMBER, 1975.

Experiments 1-6. Total Number of Bees/Plot Versus Fumigation Level (Theoretical Levels)

| | CONTROL | LOW | MEDIUM | HIGH | $X^2$ | P |
|---|---|---|---|---|---|---|
| Number of Bees | 91 | 118 | 127 | 109 | 6.38 | >0.05 |

Experiments 3 and 4. Total Number of Bees/Plot at Dishes a) Hidden in Grass with No Fragrance; b) Hidden with Essential Oil Fragrance; c) Not Hidden, e.g., white 20 cm squares under dishes. (30 minute tests).

| | | CONTROL | LOW | MEDIUM | HIGH |
|---|---|---|---|---|---|
| Number of Bees | a) | 0 | 0 | 0 | 0 |
| | b) | 1 | 3 | 0 | 0 |
| | c) | 172 | 124 | 169 | 120 |

During both 1975 and 1976, mortality of the bees at the ZAPS site appeared to be normal; no obvious lethal effects could be discerned. It should be noted that the hives were not placed directly on the fumigated plots primarily because of concern expressed about possible problems associated with honeybees and researchers in close proximity.

The bees did not appear to avoid different concentrations of $SO_2$ gas nor did they exhibit obvious signs of intoxication, either lethal or sublethal, such as interference with foraging activity. But in 1975, the bees did not successfully locate feeding stations hidden from view, even if a fragrance was added. Odor may be more important than vision as a guidance system to bees, and the addition of small amounts of an essential oil should be a strong attractant (von Frisch, 1967). It is possible that the $SO_2$ interfered with the bees' olfactory sensory systems or that other odors are more attractive than peppermint.

The long-term effects of continued exposure to $SO_2$ are still not known, but it appeared that during the summers of 1975 and 1976, a large portion of the bees from each colony did not forage on the control or fumigated plots and as such, may have had little contact with the $SO_2$.

Acetylcholinesterase (Ache) enzyme analyses on heads or brains from bees (both foraging and dying bees), which were presented in the portion of this report describing the chemical analyses of bee tissue from sites throughout southeastern Montana, were performed by R. Barker, Bee Research Laboratory, Tucson, Arizona. He found that the rates of enzyme activity of bees from the ZAPS I site and controls from apiaries near Broadus, Montana, were as high or higher than the normal activity rates of bees from the Tucson area and considerably higher than the rates of enzyme-activity in bees poisoned by organo-phosphate pesticides. Failure to detect an $SO_2$-induced decrease in the Ache activity of bees at the ZAPS sites may be due to avoidance of the $SO_2$ since the hives were not placed on the treatment plots and the bees were free to forage elsewhere. Foraging outside of the ZAPS plots may have been related to a lack of good forage materials on the plots rather than avoidance of the sulfur, which from the feeding tests appeared to be the case.

During 1976, the queens from each hive, except the one above the LOW plot, superceded, leaving daughter queens to take their places. However, the hive above the MEDIUM plot was left in a queenless state. This colony was replaced in late June with a colony from the Broadus area and an extra colony was set above the control plot to use in case of additional supercedures. Because of the supercedures, in the fall the strength of the colonies and the amount of stored honey ranked from the largest to smallest was: LOW, CONTROL, MEDIUM, HIGH.

In late September of 1976, honeybees began to seek out the drop-traps for water, as indicated by their lapping of water from the sides of the trap. The result was that bees were captured in these traps at this time of the year when they had not been captured before. For example, by walking a transect across the plots, the numbers of bees on the plots near these traps (September 21, 1976) was 31, 25, 2, and 2 for the CONTROL, LOW, MEDIUM, and HIGH treatments, respectively. However, because of the supercedures, these numbers approximated the difference in the strength and size of the colonies nearest each plot.

Bees from the ZAPS I site in 1975 had slightly lower sulfur content ($\bar{x} = 4,000$, SD = 436) than those obtained in the previous year near Colstrip ($\bar{x} = 4,392$, SD = 286). In 1976, bees from ZAPS I had lower sulfur ($\bar{x} = 2,508$, SD = 504) in late June than in August ($\bar{x} = 3,990$, SD = 942). However, in August, the bees near Colstrip contained more sulfur ($\bar{x} = 4,933$, SD = 506) than bees from ZAPS. Hillmann (1972) found lower sulfur in larval bees than in adult bees. In June, bee colonies would have contained a greater proportion of young bees than the same colonies in August.

Currently, the drop-trap captures are being processed, and those data will be presented in the next report. The traps worked well and were very specific to the collection of bees and flies. The pollen they were carrying verified them as pollinators.

We also are in the process of completing the sticky trap evaluations. So far, we have not found any demonstrable trends in numbers across the plots. Yellow sulfur butterflies (Colias eurytheme Bdv.) were attracted to yellow sticky traps over white traps. The numbers were significantly different for some plots (Table 14.3) but did not correlate to the $SO_2$ treatment levels.

The deposition of small insects on the sticky traps may be useful for the interpretation of prevailing wind patterns on the plots at the level of the pipeline, assuming that the insects are deposited more or less as inert particles as suggested by Taylor (1962). Wind roses, based on insect deposition for each plot, are presented in Appendix C. This information has been included because the instrumentated wind data are not yet available from EPA, and because there is only a single wind monitor at each of the ZAPS sites, and it is located approximately three meters above the surrounding terrain and may not produce readings representative of wind patterns at the level of the pipes. The insects counted on the traps for this purpose were small, weak fliers such as aphids and thrips, which would most likely be active during the daylight hours.

To date, the identifications and counts of beetles in the pit-fall traps have been completed for about 80 percent of the traps. Figure 14.3 presents linear regression lines (based on theoretical fumigation means) which depict the population changes of beetles of the genus Canthon (mostly Canthon laevis), a decomposer dependent on fecal and dead organic materials (Ritcher, 1958). The trapping data (Appendix D) for May at ZAPS I versus ZAPS II shows that more beetles were present on each of the ZAPS II plots. The number of beetles captured on ZAPS I in May was low compared to ZAPS II, but the population numbers increased until they were only slightly lower than those at ZAPS II in August. Also, the number of beetles captured on the LOW plot at ZAPS I in May was unusually low (21) compared to other plots and dates, which produced a positive regression slope; but the number of the beetles on the CONTROL plot exceeded those at any other ZAPS I plot. Computing a regression line for the number of beetles on the CONTROL, MEDIUM and HIGH plots gave a negative slope, which is still less steep than that for ZAPS II in May.

The relatively low beetle populations on ZAPS I in May could be due to a residual effect from the previous year's fumigations, to $SO_2$ levels above the desired delivery concentrations on some of the plots during the spring equipment adjustments, to grazing influences (ZAPS II was fenced in 1976 and ZAPS I in 1975) or to attraction of beetles to rodents caught in traps which had inadvertantly been left on ZAPS I during the first part of May.

TABLE 14.3. STICKY-TRAP CAPTURE OF COLIAS EURYTHEME (SULFUR BUTTERFLIES) FOR 1520 TRAP DAYS, FIVE SAMPLE PERIODS, N = 985.

| | ZAPS I $SO_2$ | | | | ZAPS II $SO_2$ | | | |
|---|---|---|---|---|---|---|---|---|
| Period Ending | CONTROL | LOW | MEDIUM | HIGH | CONTROL | LOW | MEDIUM | HIGH |
| July 19, 1976 | 35 | 18 | 28 | 24 | 26 | 28 | 21 | 54 |
| Aug. 5, 1976 | 50 | 55 | 27 | 35 | 26 | 25 | 41 | 36 |
| Aug. 16, 1976 | 18 | 26 | 29 | 22 | 43 | 44 | 29 | 38 |
| Sept. 2, 1976 | 10 | 9 | 7 | 19 | 19 | 13 | 10 | 13 |
| Sept. 19, 1976 | 14 | 3 | 12 | 10 | 27 | 13 | 8 | 20 |
| Mean number Total Capture | 25.4 | 22.2 | 20.6 | 20.0 | 28.2 | 24.6 | 21.8 | 32.2 |
| SD | 16.7 | 20.3 | 10.3 | 9.0 | 8.9 | 12.8 | 13.7 | 16.1 |
| SE | 7.5 | 9.1 | 4.6 | 4.0 | 4.0 | 5.7 | 6.1 | 7.2 |
| Goodness of Fit Total Capture | $X^2_I = 2.7$, df = 3, $P > 0.4$ | | | | $X^2_{II} = 9.6$, df = 3, $P < 0.05$ | | | |
| Plot by Plot | $X^2_{II} = 109.9$, df = 1, $P < 0.001$<br>for HIGH vs MEDIUM | | | | $X^2_{II} = 5.0$, df = 1, $P < 0.05$<br>for HIGH vs LOW | | | |
| | $X^2_{II} = 5.1$, df = 1, $P < 0.05$<br>for MEDIUM vs CONTROL | | | | $X^2_{II} = 1.3$, df = 1, $P > 0.3$<br>for CONTROL vs HIGH | | | |

By August, the Canthon beetle populations on the control plots of both ZAPS I and II were very similar, and the regression slopes describing the population decreases across the plots at both sites were almost identical, indicating that the populations of this beetle were influenced by the increasing $SO_2$ concentrations. We have not presented significance levels for regression because at the time of this report data concerning the measured levels of $SO_2$ in the ambient air were not available.

The results of chi square analyses to test significance of the different total catches for each plot were highly significant (Appendix D). This conclusion is consistent with that of Frietag et al. (1973), who reported a negative correlation between the numbers of ground beetles (primarily predatory Carabids and one carrion-feeding Silphid) and sulfate fallout from a Kraft mill.

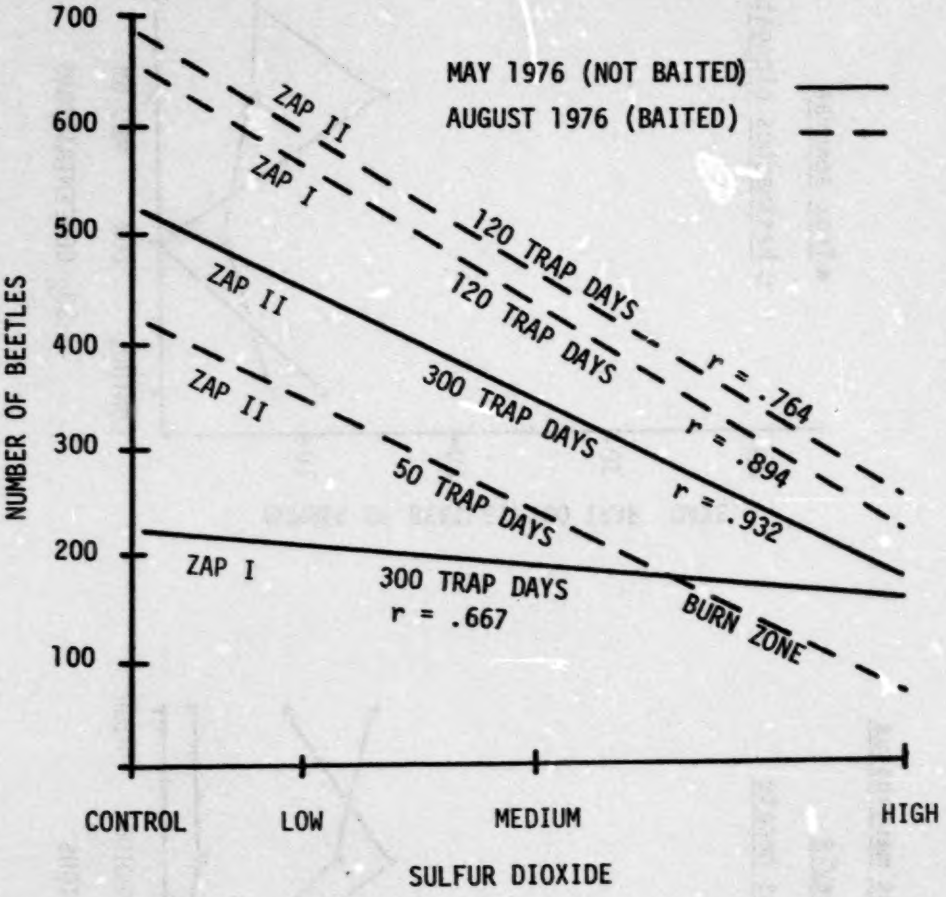

Figure 14.3. Number of beetles (Canthon sp.) in pit-fall traps/$SO_2$ treatment plot, plotted against theoretical fumigation levels.

In May, significant differences were documented ($X^2$ analysis) (Appendix E) at both sites in the numbers of five species of beetles representing three families (Carabids, Silphids, and Scarabaeids). In most cases, the number of beetles tended to decline sharply (Figure 14.4) as the $SO_2$ concentration increased. Later the capture numbers of all but the Canthon beetles and of the Silphids (Nicrophorus spp.) decreased to very low levels on all plots, including the controls, and the numbers of Silphids no longer showed any demonstrable relation to $SO_2$ level ($X^2$ analyses).

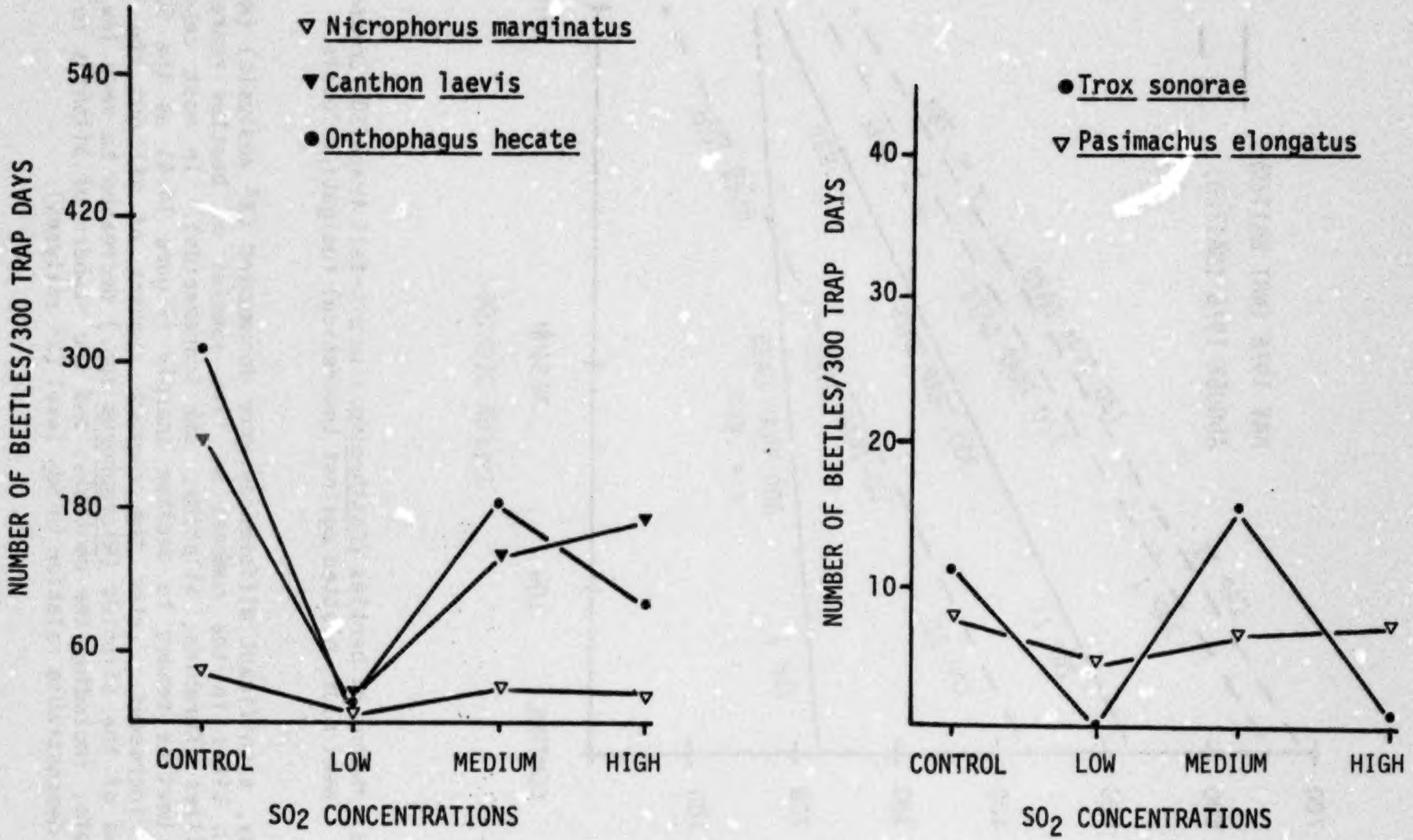

Figure 14.4A. Pit-fall captures of beetles on ZAPS I treatment plots, May 1976.

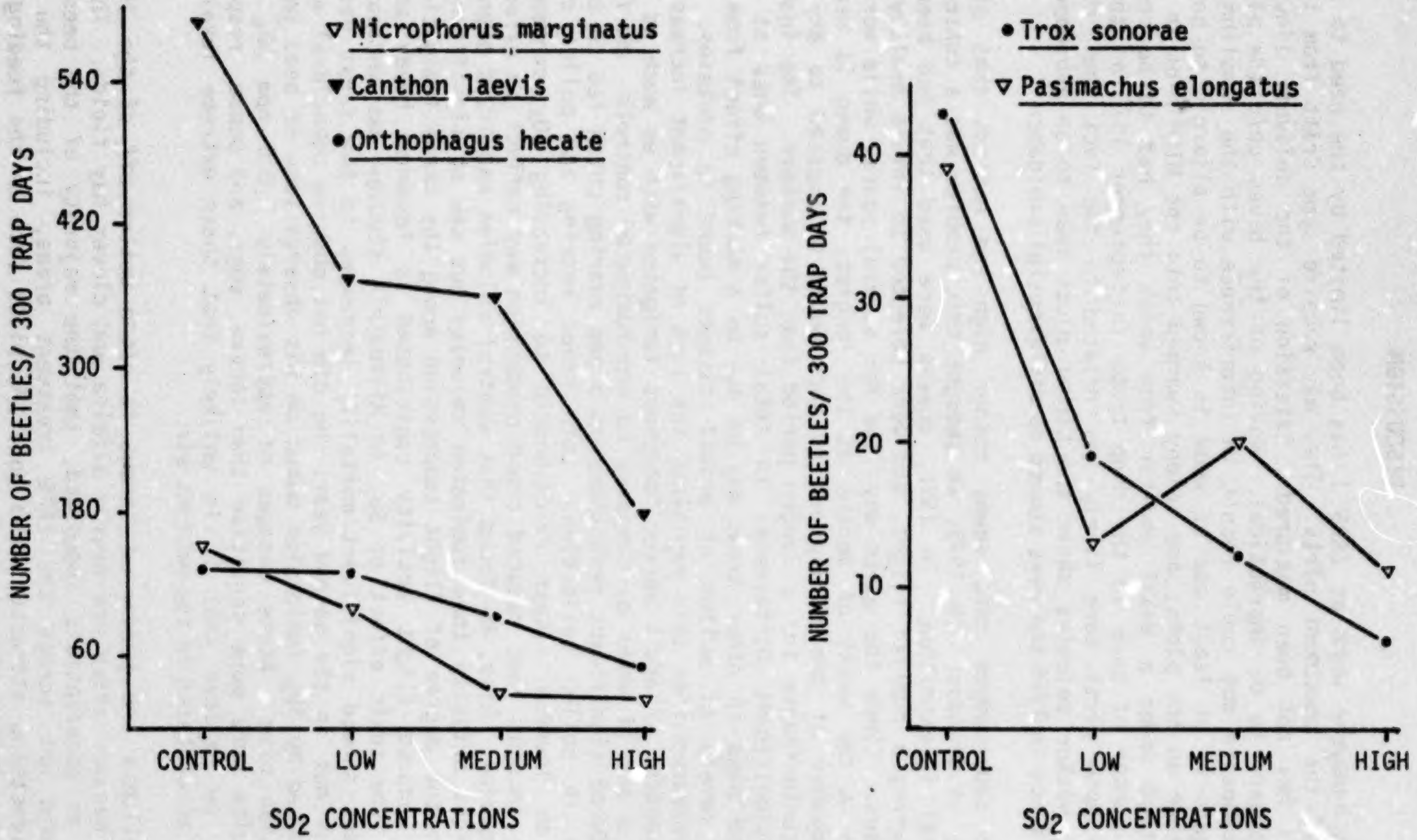

Figure 14.4B. Pit-fall captures of beetles on ZAPS II treatment plots, May 1976.

# DISCUSSION

The honeybee work at ZAPS I has been limited by the need to place the hives off the treatment plots. They may receive some drift from the plots, but this has not been measured. Extension of the delivery lines to the hives appears to be impractical. Moving of the hives onto the plots would insure exposure but could result in interference with the sampling by other investigators, at least one of whom is known to be allergic to bee stings. Bees forage on the plots; one colony swarmed onto the HIGH plot in June 1976 and settled under a small shelter from which they had to be removed. A greater number of bees in the drop traps in September 1976 on the CONTROL and LOW pphm plots more likely was related to the fact that there were smaller, weaker colonies above the higher plots than to an avoidance of the sulfur, since in 1975 the bees showed no differential avoidance.

The supercedure rate seems rather high and exceeds that of similar colonies in Broadus. In 1975, we thought this problem was a consequence of artificial insemination. In 1976, queens were used that had been inseminated during a nuptial flight, and were selected to insure healthy colonies and queens. Since the queen may live for several years, while workers only live for a few weeks or months at the longest, the queen is usually the oldest member of the colony and as such would be exposed to any environmental contaminants for a longer period than the workers. The inability to detect significant differences in total sulfur between bees at the ZAPS plots and bees in other areas may be due to a masking effect from the high natural levels of sulfur of animal tissues bound in proteins. Hillmann (1972) reasoned that this explained the lack of significant increases in the sulfur content of adult worker honeybees fumigated with as much as 5 pphm of $SO_2$ for 9 and 14 weeks as compared to non-fumigated controls. He found that $SO_2$ produced significant reductions in brood rearing which led to correlated declines in pollen collection. Both brood rearing and pollen collection showed an inverse linear relationship to increasing $SO_2$ concentrations. Hillmann thought that reduced brood production may reflect an effect of $SO_2$ on the queens. Also, he found that control colonies exhibited significantly more flight activity than fumigated colonies but saw no significant differences in the degree of flight suppression among the three fumigation treatments. Reduced flight activity contributed to lessened honey and colony weight. The toxic effect of $SO_2$ in Hillmann's studies was unclear: fumigated bees showed significant mortality increases in the first year of the study but not in the second year. He did not observe behavioral abnormalities induced by $SO_2$ fumigation based on his observations of bees in a glass-observation hive. Acute dosages of approximately 1,000 ppm $SO_2$ indicated that adults were more sensitive than larvae, eggs, and pupae, respectively. However, we believe that it is unlikely that these extreme levels of $SO_2$ would be encountered in the ambient air.

Available and preferred forage is often better off of the ZAPS plots than on because there are nearby alfalfa and clover hay fields. Thus, it is likely, as observations suggested, that the majority of the bees in each colony did not forage the ZAPS treatment areas, including the control, unless something attracted them onto the plots such as the feeding stations or the water in the drop-traps.

Since significant increases in fluorides in bees were found near Colstrip and a probable toxic effect was identified at one site (see Colstrip vicinity studies, this report), it is believed that honeybees are particularily efficient and sensitive bioindicators of some major and trace elements. Sulfur and sulfur compound impacts may be more difficult to identify and measure, but Hillmann's data (1972) indicate that sulfur dioxide does have significant impacts on honeybees. In 1977, we intend to move the bees into areas where the bees may have a greater probability of exposure to $SO_2$, such as in the buffer zones, and the levels to which they are exposed using sulfation plates will be monitored at each hive. Behavioral responses such as flight activity and physiological responses such as mortality and brood rearing influenced by $SO_2$ will be examined. For comparison purposes, techniques will be similar to those used by Hillmann.

Hillmann (1972) found that tissues of larval or immature bees had significantly less sulfur than those of adult bees. The bees at the ZAPS plots contained less sulfur in June than in August. June is a period of rapid population increase; whereas August is a period of a relatively stable or declining population size. Thus, the bee populations in June would have had a greater proportion of young adult bees, which probably accounted for the lower sulfur content. Bees at the ZAPS plots consistently demonstrated lower sulfur levels than bees at the other sites. Also, there seemed to be a tendency towards lower sulfur in both bees and beetles on the HIGH treatment plot. Lower sulfur content in insects on plots fumigated with $SO_2$ could occur as a consequence of rapid population turnover or shortened life span. This would increase the proportion of young to old members of the population. Assuming that sulfur increases in older insects, this could explain the observed trend.

Data from the beetle trapping are important as regards soil cycles. The Scarabaeidae and the Silphidae gather, bury, and break down organic material. This in turn releases nutrients to the soil systems. There are several literature reports that support the hypothesis that air pollutants, particularly heavy metals, accumulate in litter and the upper soil horizons and interfere with nutrient cycling. In a review on depressed litter decomposition and reduced nutrient availability, Smith (1975) stressed the need for studies on the effects of contaminants on these processes and their ultimate effect on plant growth. Also, many contaminants are toxic to microorganisms, particularly fungi, which are primarily responsible for the decomposition of organic materials in the soil (Horsfall, 1956). A number of insects, including the dung beetles such as Canthon, disperse spores of saprophagic or coprophilous fungi (Lodha, 1974). These insects act both as agents of mechanical breakdown and movement of organic materials and as disseminators of primary microbial decomposers. Thus, any reduction in the populations of these beetles could have effects on the decomposition and nutrient cycles.

Based on the data, the numbers of Canthon beetles on ZAPS I built up through the summer to the level of the beetles on the ZAPS II plots. This indicates that what may be occurring is a depressed immigration or population growth on the fumigated plots rather than an elimination or migration of the beetles from these plots. Waldow (1973) discovered an antennal cell, a coelosphaerica, in carrion beetles (Nicrophorus vespilloides) which responds to the odor of carrion, $H_2S$, and a few cyclic compounds. This receptor may be responsible for a coding of the odor of rotten meat. Sulfur dioxide could act in a somewhat similar manner, interacting on the insects' ability to orient to food material by olfaction. This can be tested in a simple y-tube olfactometer patterned on the design by Burkholder (1970).

Data from the other sampling procedures at ZAPS as well as within-plot distributions of insects, will be presented in the next interim report.

## SUMMARY AND CONCLUSIONS

We have focused our studies on ground-dwelling beetles and on honeybees--insects which our studies and those of the Colorado investigators indicate are most susceptible to $SO_2$.

Both the types of experiments and the results of experiments using honeybees at the ZAPS plots were limited by the placement of the beehives 75 m above each of the treatment plots. This averted possible problems for investigators from bees in close proximity. However, it introduced a new problem since only bees foraging on the plots would be expected to receive a full strength exposure of short duration to the $SO_2$ gas. The bees did not demonstrate measurable avoidance of the sulfur gas on any of the plots, nor did they exhibit obvious signs of sublethal effects such as intoxication or of lethal effects as indicated by mortality. However, bees did not successfully locate feeding stations hidden from view, even if an attractive fragrance was added to the sugar solution. Odor may be more important than vision as a guidance system to bees, and the addition of small amounts of essential oils should be a strong attractant (von Frisch, 1967). It is possible that the $SO_2$ interfered with the bees' olfactory sensory systems or that odors other than those tested are more attractive.

In May, several species of ground-dwelling beetles, mostly Scarabaeids and Silphids (carrion beetles), demonstrated significant inverse relationships in numbers compared to $SO_2$ fumigation concentration. By late summer, tumblebugs (Canthon spp.) continued to demonstrate this relationship. The response of carrion feeders was variable, and the capture of other beetle species was too low for meaningful comparisons. The inverse relationships of numbers to $SO_2$ levels appeared to be related to movement of beetles onto the plots, since the beetle numbers on the ZAPS I plots increased during the season. The increase was greatest on the control and the low fumigation treatments. Hydrogen sulfide apparently is an attractant to carrion beetles (Waldow, 1973). Since hydrogen sulfide attracts carrion beetles, it was hypothesized that other sulfur compounds may act in a similar manner, and the release of $SO_2$ gas over large areas could mask the ability of beetles to detect and to orient towards food sources of decaying organic materials.

The sulfur content in honeybees reared at the ZAPS plots during 1975 and 1976 tended to be somewhat lower than the sulfur content of bees at sites in other areas of southeastern Montana. Also, the sulfur content of bees at ZAPS was relatively low in June and increased by August 1976. In both bees and beetles, there appeared to be a trend towards decreased sulfur in insects on the HIGH treatment plots. Since Hillmann (1972) reported that immature bees had significantly less sulfur than older bees, it was suggested that decreased sulfur in insects exposed to $SO_2$ could be explained by high turnovers or shortened life spans of the members of a population, which would result in a higher proportion of younger to older individuals.

The data processing and analyses of insects other than beetles captured by the trapping systems (pit-fall, drop-trap, and sticky-trap) in 1976 were not complete at the time of this report, but this information should be available for the next interim report.

Emphasis on sampling programs (population estimates and population dispersal) will be continued but will be restricted to selected species and refined to maximize trapping or capture precision and success, applicability to statistical analyses, and efficiency in terms of man-hours. Trapping regimens will be limited to three or four periods coinciding with seasonal population cycles, replacing more or less continuous sampling at considerable effort and expense. The intent is to de-emphasize repetitive sampling and redirect efforts toward process- and mechanism-oriented experiments to determine the basis for observed responses to $SO_2$.

At present, beetles seem to offer the greatest potential for studies at the ZAPS plots. Not only do they appear to respond to $SO_2$ but they include important predators, saprophages, and necrophages. The latter two groups undoubtedly play a role in litter decomposition and nutrient cycling. Rigorous investigations of one or two major species of beetles and of the major ecological events and processes interacting with these populations are needed. Also, the role of these species in the grassland ecosystem and soil ecosystem must be appraised. This requires both quantitative and qualitative information regarding behavior, seasonal cycles, and population dynamics relative to changes in biotic and abiotic components of the ecosystems in order to evaluate the chronic effects of $SO_2$ stress. This work would contribute both to the goals of the overall project as regards the impact of $SO_2$ and would provide valuable information concerning soil systems and the ecology of soil animals. It is particularly well-suited to the current needs of the project in view of a lack of information on soil systems and of the cessation of above-ground arthropod sampling and re-directed emphasis by the Colorado investigators on soil arthropods.

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# APPENDIX A

See Appendix A in the section 5 of this report by Bromenshenk entitled:

## INVESTIGATION OF THE IMPACT OF COAL-FIRED POWER PLANT EMISSIONS UPON INSECTS: ENTOMOLOGICAL STUDIES IN THE VICINITY OF COLSTRIP, MONTANA

# APPENDIX B

## DESCRIPTIONS OF THE INBRED LINES OF HONEYBEES UTILIZED IN THE ARTIFICIALLY INSEMINATED QUEENS OBTAINED FROM THE USDA BEE STOCK CENTER.

Queens were a dihybrid YDGk.

Drones were a Pa line.

YD is a yellow line. Queens are stocky. Brood rearing is initiated early in the season and continues late into the fall. They winter poorly, consuming lots of honey and often producing inadequate stores.

Gk is an intermediate color between black and yellow. The workers are small. The queens do not lay eggs well. Honey production is low. Wintering is poor. Workers are marked by 1 or 2 yellow bands.

YDGk hybrids produce a good line.

Pa is a yellow line. The queens are large and are the best layers of the 18 lines at the Bee Stock Center. Brood rearing begins early and ends late in the season. Population size is good during the foraging season. The bees store little honey and are very susceptible to American Foul Brood.

YDGkxPa makes a good cross.

## APPENDIX C

Wind roses derived from the deposition of wind-carried insects on sticky traps. The length of each line indicates the relative frequency of insects captured and as such the prevailing wind pattern during the daylight hours. The angle indicates wind direction relative to the layout of the plots, thus a horizontal line indicates wind current perpendicular to the N-S axis of the plots. In all cases the lines indicate wind directed towards the point of intersection.

ZAPS I WIND ROSES, JULY 15, 1976

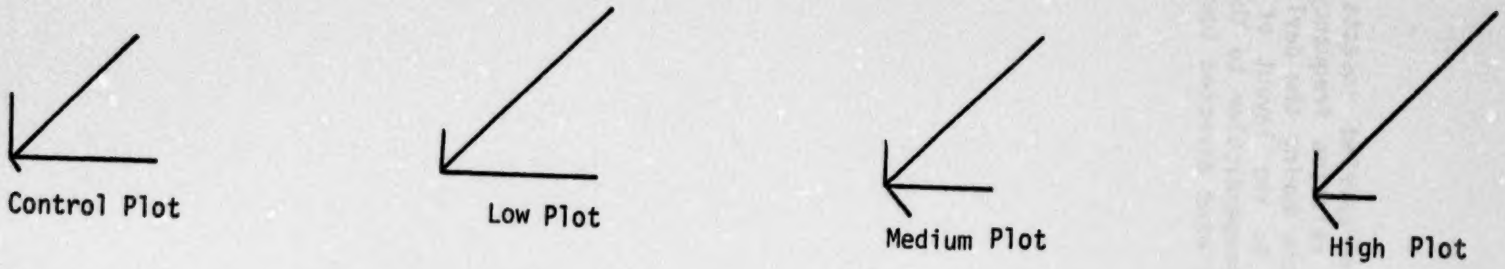


Figure C-1.

ZAPS I WIND ROSES, AUGUST 5, 1976

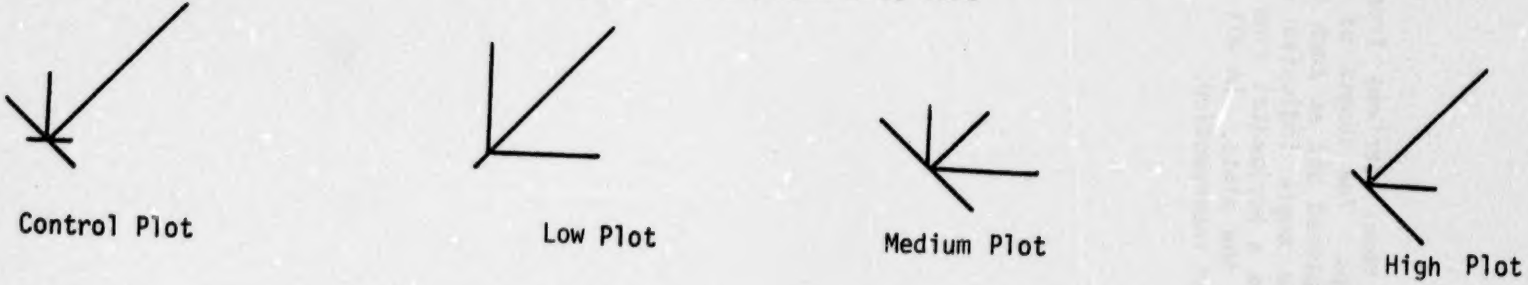
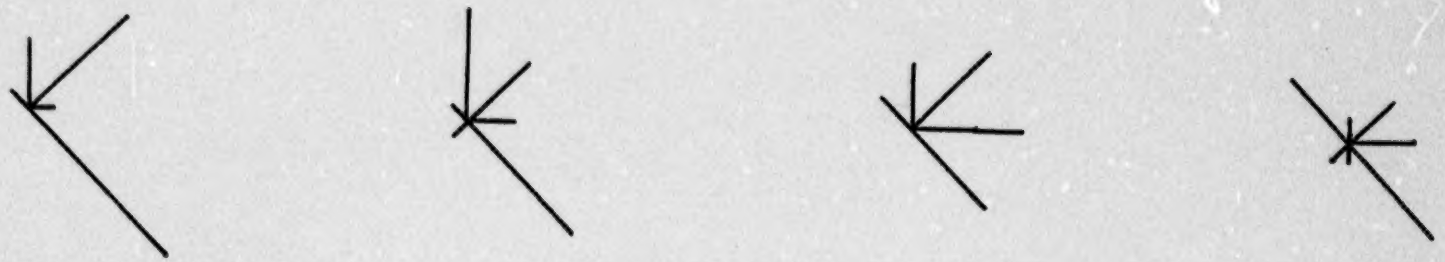


Figure C-2.

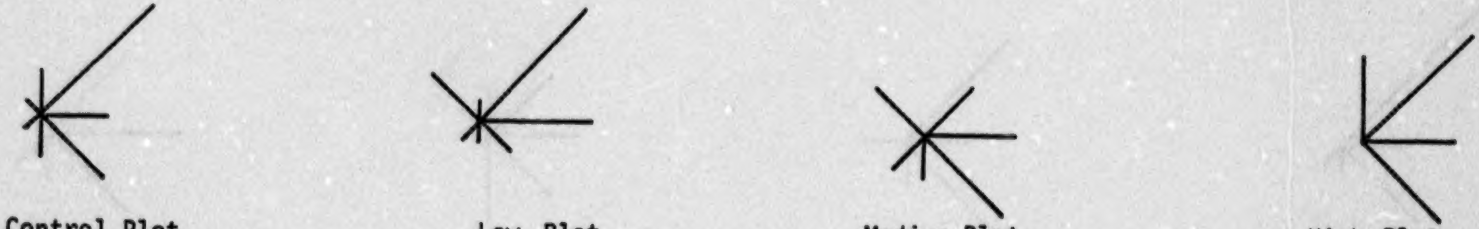
ZAPS I WIND ROSES, AUGUST 16, 1976



Control Plot  Low Plot  Medium Plot  High Plot

Figure C-3.

ZAPS I WIND ROSES, SEPTEMBER 2, 1976



Control Plot  Low Plot  Medium Plot  High Plot

Figure C-4.

ZAPS I WIND ROSES, SEPTEMBER 19, 1976

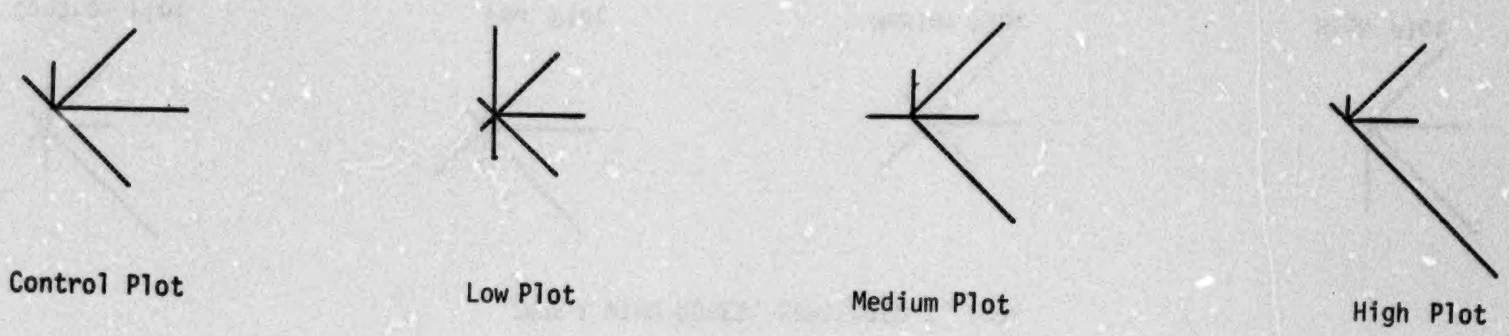


Figure C-5.

ZAPS II WIND ROSES, JULY 15, 1976

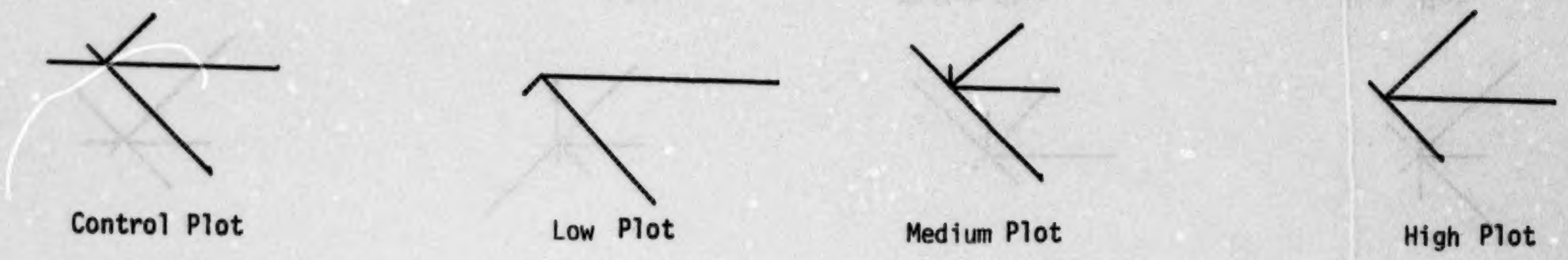


Figure C-6

ZAPS II WIND ROSES, AUGUST 5, 1976

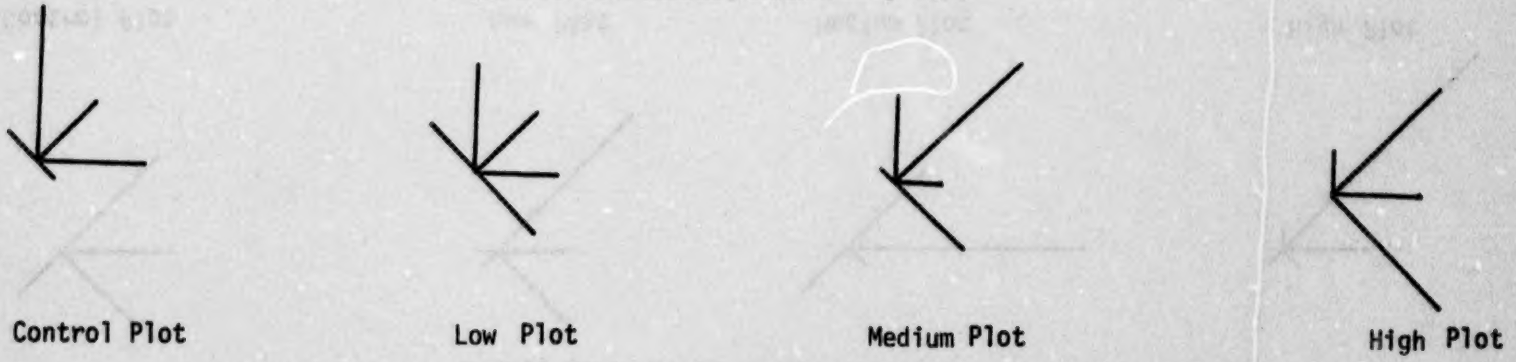


Figure C-7.

ZAPS II WIND ROSES, AUGUST 16, 1976

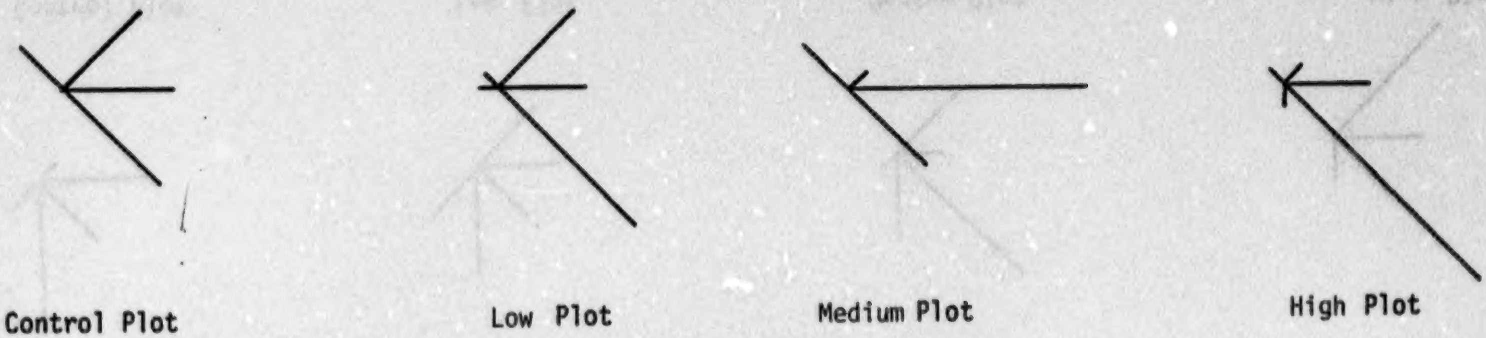


Figure C-8.

ZAPS II WIND ROSES, SEPTEMBER 2, 1976

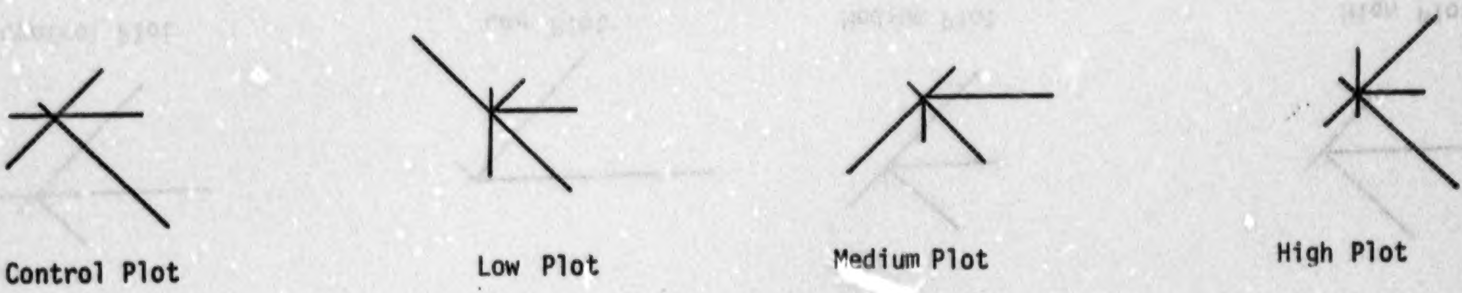


Figure C-9.

ZAPS II WIND ROSES, SEPTEMBER 19, 1976

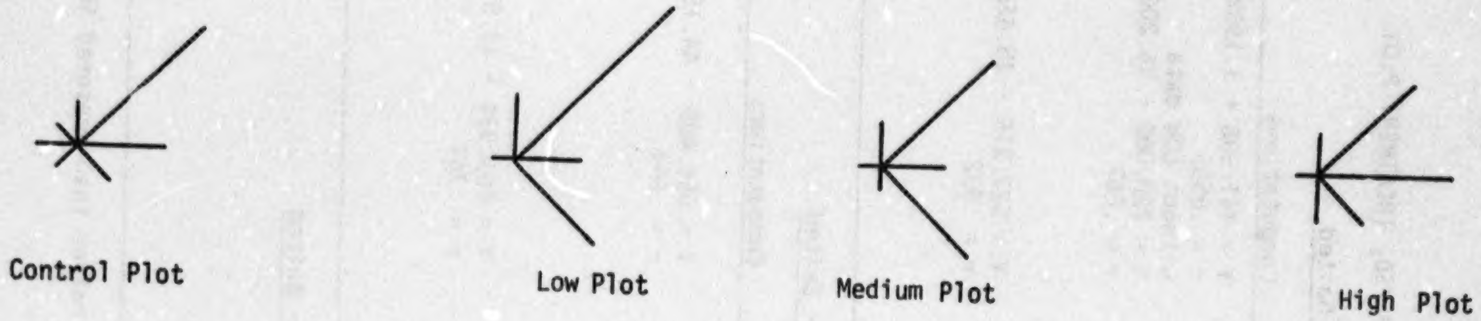


Figure C-10.

## APPENDIX D

## NUMBER OF BEETLES (CANTHON SP.) IN PIT-FALL TRAPS/$SO_2$ TREATMENT PLOT.

May 1976--300 trap days/plot--not baited

| Site | | Number of Beetles | Computations |
|---|---|---|---|
| ZAPS I | CONTROL | 240 | Y = 141.908 + 1.198X |
| | LOW | 21 | r = .0565 |
| | MEDIUM | 149 | without LOW data |
| | HIGH | 148 | Y = 220.000 - 16.200X |
| | All plots | 558 | r = .667 |
| | $X^2$ = 174.2, df=3, P<0.005 | | |
| ZAPS II | CONTROL | 589 | |
| | LOW | 374 | Y = 527.216 - 35.426X |
| | MEDIUM | 358 | r = .932 |
| | HIGH | 185 | |
| | All plots | 1506 | |
| | $X^2$ = 218.3, df=3, P<0.005 | | |

August 1976 - 120 trap days/plot - Baited

| Site | | Number of Beetles | Computations |
|---|---|---|---|
| ZAPS I | CONTROL | 767 | |
| | LOW | 476 | Y = 658.405 - 44.[illegible]54 |
| | MEDIUM | 371 | r = .894 |
| | HIGH | 269 | |
| | All plots | 1883 | |
| | $X^2$ = 294.1, df=3, P<0.005 | | |
| ZAPS II | CONTROL | 864 | |
| | LOW | 443 | Y = 683.934 - 43.573X |
| | MEDIUM | 352 | r = .764 |
| | HIGH | 336 | |
| | All plots | 1995 | |
| | $X^2$ = 370.0, df=3, P<0.005 | | |

August 1976 - 50 trap days/plot - Baited
(Vegetation Burn Areas)

| | |
|---|---|
| CONTROL | 426 |
| HIGH | 66 |
| $X^2$ = 263.4, df=3, P<0.005 | |

*Regression computations are based on theoretical rather than measured $SO_2$ delivery concentrations.

## APPENDIX E

### $X^2$ EVALUATION (EXPECTED VALUES EQUAL) BEETLE CAPTURES ON ZAPS I AND II TREATMENT PLOTS IN MAY 1976.

| | Plot | | | | | |
|---|---|---|---|---|---|---|
| Species | CONTROL | LOW | MEDIUM | HIGH | $X^2$ | P |
| | | | ZAPS I | | | |
| Canthon sp. | 589 | 374 | 358 | 185 | 218.3 | $P<0.005$ |
| Nicrophorus sp. | 153 | 100 | 26 | 21 | 160.4 | $P<0.005$ |
| Onthophagus sp. | 136 | 131 | 92 | 49 | 48.1 | $P<0.005$ |
| Trox sp. | 43 | 19 | 12 | 6 | 39.5 | $P<0.005$ |
| Pasimachus sp. | 39 | 13 | 20 | 11 | 23.6 | $P<0.005$ |
| | | | ZAPS II | | | |
| Canthon sp. | 240 | 21 | 149 | 178 | 173.4 | $P<0.005$ |
| Nicrophorus sp. | 38 | 9 | 32 | 20 | 20.15 | $P<0.005$ |
| Onthophagus sp. | 315 | 22 | 189 | 109 | 292.9 | $P<0.005$ |
| Trox sp | 12 | 0 | 17 | 1 | 27.9 | $P<0.005$ |
| Pasimachus sp. | 8 | 5 | 7 | 8 | .86 | $P<0.5$ |

## SECTION 15

# THE RELATIVE SENSITIVITY OF SELECTED PLANT SPECIES TO SEVERAL POLLUTANTS SINGLY AND IN COMBINATION

by

D. T. Tingey, L. Bard, and R. W. Field

## INTRODUCTION

With the introduction of coal-fired power plants into grassland ecosystems it becomes important to recognize and determine the possible effects of power plant emissions on the ecosystems. There have been only limited studies on native prairie plant species either describing injury symptoms or providing relative sensitivity data (Davis et al., 1966; Hill et al., 1974).

The objectives of this study were to describe the air pollution-induced symptoms and to determine the relative sensitivity of selected plant species to common air pollutants, singly and in combination.

## METHODS AND MATERIALS

### PLANT GROWTH

Plant growth and exposures to air pollutants were conducted in greenhouse facilities in Corvallis, Oregon. The plant material used in the study (Table 15.1) was collected in the Otter Creek Valley, Montana, near the Fort Howes Ranger Station during August, 1974. Since then, the individual species were propagated vegetatively; the grasses by division and Fringed Sage Wort by cuttings. The plants were grown in 225 ml styrofoam cups containing a 2:1 (v:v) mixture of perlite:Jiffy Mix. Plants for all studies were grown in greenhouses; watered daily with North Carolina State University phytotron nutrient solution (Downs and Hellmers, 1975) and periodically leached with water. The plants were grown at day/night temperatures of 26-32/18¼C; the sunlight was supplemented and the light period extended to 16 hr/day with light from HID sodium vapor lamps. Maximum light intensities were 450-650 microeinstenins $M^{-2}$ sec$^{-1}$.

### PLANT EXPOSURES

Pollutant exposures were conducted in single pass exposure chambers located in a greenhouse (Heck, Dunning, and Johnson, 1968). Sulfur dioxide and nitrogen dioxide diluted in nitrogen were metered into the exposure chambers at a rate sufficient to maintain the desired gas phase concentrations. Sulfur

TABLE 15.1. PERCENT FOLIAR INJURY OF PLANTS EXPOSED TO SULFUR DIOXIDE FOR 4 HOURS[1]

| Plant Species | $SO_2$ Conc. (ppm) | | | |
|---|---|---|---|---|
| | 0.5 | 1.0 | 1.5 | 2.0 |
| *Agropyron smithii* Rydb<br>WESTERN WHEAT GRASS | 0 | 0 | 0 | 3 |
| *Bouteloua gracilis* Lag.<br>BLUE GRAMMA | 0 | 3 | 3 | 6 |
| *Koeleria cristata* (L.) Pers.<br>PRAIRIE JUNE GRASS | 0 | 0 | 1 | 6 |
| *Stipa comata* Trin. and Rupr.<br>NEEDLE & THREAD GRASS | 0 | 0 | 0 | 2 |
| *Artemisia frigida* Willd.<br>FRINGED SAGE WORT | 0 | 0 | 6 | 13 |
| *Triticum aestivium* L. em Thell.<br>WHEAT, HYSLOP | 0 | 0 | 4 | 5 |

[1] Plants were exposed for 4 hr and foliar injury was assessed 96 hr after exposure. The injury was measured as the percentage of the leaf area showing $SO_2$ injury. Each mean was the average of 9 observations: $S_{\bar{x}} = 1$.

TABLE 15.2. PERCENT FOLIAR INJURY OF PLANTS EXPOSED TO NITROGEN DIOXIDE FOR 4 HR[1]

| Plant Species | $NO_2$ Conc. (ppm) | | | |
|---|---|---|---|---|
| | 0.5 | 1.0 | 2.0 | 4.0 |
| WESTERN WHEAT GRASS | 0 | 0 | 1 | 1 |
| BLUE GRAMMA | 0 | 3 | 4 | 8 |
| PRAIRIE JUNE GRASS | 0 | 0 | 1 | 4 |
| NEEDLE & THREAD GRASS | 0 | 0 | 1 | 1 |
| FRINGED SAGE WORT | 0 | 0 | 0 | 0 |
| WHEAT, HYSLOP | 0 | 0 | 0 | 1 |

[1] Plants were exposed for 4 hr and foliar injury was assessed 96 hr after exposure. The injury was measured as the percentage of the leaf area showing $NO_2$ injury. Each mean was the average of 9 observations: $S_{\bar{x}} = 1$.

dioxide was measured with a flame photometric sulfur dioxide analyzer; nitrogen dioxide and ozone were monitored with chemiluminescence analyzers. Prior to each exposure the analyzers were calibrated using known concentrations of each pollutant. The plants were exposed to the pollutant 4 to 6 weeks after vegetative propogation. Plant injury was visually assessed in 5% increments 96 hours following exposure as the percentage of the plant leaf area exhibiting visual pollutant effects.

## RESULTS

### PLANT INJURY DESCRIPTIONS

Plant injury resulting from sulfur dioxide, nitrogen dioxide and ozone was generally similar to previous reports (Jacobson and Hill, 1970). Injury from sulfur dioxide and nitrogen dioxide was similar on the monocots. On young leaves, injury developed at the leaf tip, on older leaves injury usually occurred at the bend of the leaf. Injury usually occurred as small bifacial lesions, and as severity increased, the lesions coalesced and spread up and down the leaf. Interveinal streaks of necrotic tissue were frequent. Lesion color ranged from light tan to ivory. On Fringed Sage Wort the injury occurred on the middleaged leaf tissue as a bifacial collapse of the tissue, killing both veins and intervenial areas. The ozone-induced injury on Fringed Sage Wort was similar to that caused by sulfur dioxide. Ozone injury to monocots appeared as tip necrosis and small necrotic lesions between the veins; as the interveinal lesions coalesced, necrotic streaking resulted. Injury tended to be concentrated between the leaf tip and bend in the leaf. Injury that resulted from the mixtures of sulfur dioxide + nitrogen dioxide or sulfur dioxide + ozone were similar to sulfur dioxide injury.

### RELATIVE SENSITIVITY

To determine the relative sensitivity of each of the plant species, they were exposed to either single pollutants or mixtures of pollutants and their injury rated. Wheat was included in all studies as an example of an agricultural crop. All species were injured following a four-hour exposure to 2 ppm $SO_2$, with Fringe Sage Wort exhibiting the most injury and Needle and Thread Grass the least (Table 15.1). Only Blue Gramma was injured at a concentration of less than 1.5 ppm.

In exposures to nitrogen dioxide (Table 15.2) only Prairie June Grass and Blue Gramma developed significant injury with Blue Gramma being more sensitive.

All plants except Needle and Thread Grass were injured by the lowest ozone concentration (0.4 ppm) (Table 15.3). Blue Gramma and wheat were the most sensitive, Western Wheat and Needle and Thread Grass the least sensitive.

Plants were exposed to either mixtures of sulfur dioxide + nitrogen dioxide (Table 15.4) or sulfur dioxide + ozone (Table 15.5) to determine if the gas mixtures interacted to alter the injury response of the plants. Injury was very similar on plants exposed to sulfur dioxide with or without nitrogen dioxide. When plants were exposed to sulfur dioxide with or without ozone,

TABLE 15.3. PERCENT FOLIAR INJURY OF PLANTS EXPOSED TO OZONE FOR 4 HR[1].

| Plant Species | $O_3$ Conc. (ppm) | | | |
|---|---|---|---|---|
| | 0.4 | 0.6 | 0.8 | 0.95 |
| WESTERN WHEAT GRASS | 1 | 2 | 5 | 7 |
| BLUE GRAMMA | 6 | 12 | 15 | 25 |
| PRAIRIE JUNE GRASS | 5 | 5 | 11 | 12 |
| NEEDLE & THREAD GRASS | 0 | 0 | 2 | 2 |
| FRINGED SAGE WORT | 2 | 4 | 11 | 12 |
| WHEAT, HYSLOP | 9 | 11 | 17 | 23 |

[1] Plants were exposed for 4 hr and foliar injury was assessed 96 hr after exposure. The injury was measured as the percentage of the leaf area showing $O_3$ injury. Each mean was the average of 9 observations, $S_{\bar{x}} = 2$.

TABLE 15.4. PERCENT FOLIAR INJURY OR PLANTS EXPOSED TO SULFUR DIOXIDE OR SULFUR DIOXIDE PLUS NITROGEN DIOXIDE[1].

| Plant Species | $SO_2$ Conc. (ppm) | | | | $SO_2$ Conc. (ppm) + 0.1 ppm $NO_2$ | | | |
|---|---|---|---|---|---|---|---|---|
| | 0.3 | 0.6 | 0.9 | 1.2 | 0.3 | 0.6 | 0.9 | 1.2 |
| WESTERN WHEAT GRASS | 0 | 0 | 2 | 4 | 0 | 3 | 4 | 5 |
| BLUE GRAMMA | 0 | 1 | 3 | 4 | 0 | 1 | 3 | 4 |
| PRAIRIE JUNE GRASS | 0 | 0 | 1 | 3 | 0 | 1 | 1 | 2 |
| NEEDLE & THREAD GRASS | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| FRINGED SAGE WORT | 0 | 0 | 1 | 4 | 0 | 0 | 0 | 0 |
| WHEAT, HYSLOP | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 |

[1] Plants were exposed for 4 hr and foliar injury was assessed 96 hr after exposure. The injury was measured as the percentage of the leaf area showing injury. Each mean was the average of 8 observations: $S_{\bar{x}} = 2$.

injury was similar except in Fringed Sage Wort where the gas mixture caused less injury than the sulfur dioxide alone.

TABLE 15.5. PERCENT FOLIAR INJURY OF PLANTS EXPOSED TO SULFUR DIOXIDE OR SULFUR DIOXIDE + OZONE[1].

| Plant Species | $SO_2$ Conc. (PPM) | | | | $SO_2$ Conc. (PPM) + 0.1 ppm $O_3$ | | | |
|---|---|---|---|---|---|---|---|---|
| | 0.5 | 1.0 | 1.5 | 2.0 | 0.5 | 1.0 | 1.5 | 2.0 |
| WESTERN WHEAT GRASS | 0 | 1 | 3 | 3 | 2 | 2 | 3 | 4 |
| BLUE GRAMMA | 1 | 2 | 2 | 2 | 1 | 3 | 2 | 4 |
| PRAIRIE JUNE GRASS | 0 | 1 | 3 | 4 | 0 | 0 | 2 | 3 |
| NEEDLE & THREAD GRASS | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 0 |
| FRINGED SAGE WORT | 0 | 9 | 24 | 33 | 0 | 0 | 4 | 6 |
| WHEAT, HYSLOP | 0 | 4 | 4 | 6 | 1 | 1 | 2 | 3 |

[1] Plants were exposed for 4 hr and foliar injury was assessed 96 hr after exposure. The injury was measured as the percentage of the leaf area showing injury. Each mean was the average of 8 observations: $S_{\bar{x}} = 1$.

## DISCUSSION

The injury observed on the native species was similar to that previously described (Jacobson and Hill, 1970; Hill *et al.*, 1974). Injury thresholds suggested for the species tested in these experiments do not take into account the effect of soil water potential on plant sensitivity. Low soil water potential could reduce plant sensitivity. The levels of pollutants used in this study are higher than would usually be expected in the field. They were used only to provide concentrations that would readily cause visual injury to aid in injury description and relative sensitivity ranking.

Davis *et al.* (1966) reported that levels of sulfur dioxide that might be expected around a Phelps Dodge smelter in Arizona, and which would defoliate cocklebur, did not injure Blue Gramma. Hill *et al.* (1974), working with established native plants in the field, showed that *Agropyron caniun* and *A. desertorum* required between 6 and 10 ppm $SO_2$ for two hours to cause visual injury. This suggests that these two species of Wheat Grass are more tolerant of $SO_2$ than Western Wheat Grass. Hill watered the plants for a month prior to exposure to ensure that they were in a stage of rapid growth and thus highly sensitive. He also reported that *Stipa occidentalis* required 10 ppm $SO_2$ for two hours to induce visual injury. This suggests that *Stipa occidentalis* is more tolerant than *Stipa comata* which showed injury at 2 ppm $SO_2$ for four hours.

Previous reports (Tingey *et al.*, 1971; Tingey *et al.*, 1973) of greenhouse studies suggested that mixtures of sulfur dioxide + nitrogen dioxide or sulfur dioxide + ozone interacted to cause more foliar injury than the effects of the single gases. These observations were not substantiated in this study. The gas mixtures did not increase the injury over that caused by sulfur dioxide alone. The lack of synergism from sulfur dioxide + nitrogen has previously been reported for native plants (Hill et al., 1974) and in growth chamber studies (Bennet et al., 1975).

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## SECTION 16

# PROGRESS IN MODELING THE EFFECTS OF $SO_2$ FUMIGATION ON AN EASTERN MONTANA GRASSLAND

by

J. L. Dodd, M. Coughenour, and W. K. Lauenroth

## INTRODUCTION

The modeling effort is charged with (1) adapting ELM (the grassland ecosystem simulation model) to the Montana sites; (2) developing and implementing simulated direct effects of atmospheric $SO_2$ on ecosystem components; and (3) developing a sulfur submodel to examine the sulfur cycle. Work is proceeding ahead of schedule in the cases of (2) and (3) and is slightly behind schedule in the case of (1).

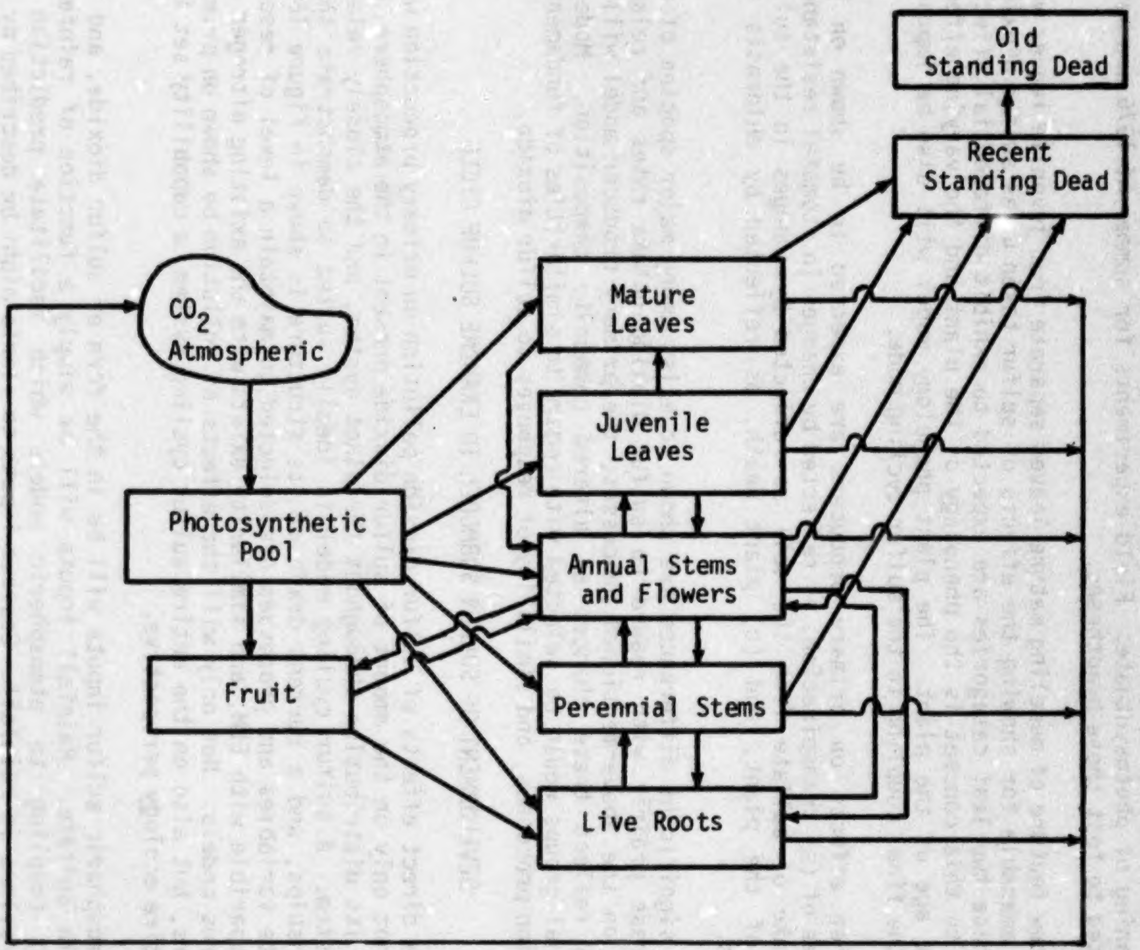
## ADAPTATION OF ELM TO LOCAL SITES

Adaptation of ELM to the mixed-grass prairie ecosystem has proceeded through completion of the application and validation of the abiotic submodel. Initial conditions, parameter values, and validation information for the producer, decomposer, nitrogen, and phosphorus submodels are completed.

The investigations are currently examining procedures for model adaptation. Information developed by the Natural Resources Ecology Laboratory (NREL) modeling team and supported by new findings of Dave Tingey (personal communication) suggest that modification of the structure of the producer submodel will lead to considerable conceptual strengthening of the whole model, especially in regard to the effects of $SO_2$ on the ecosystem. The dilemma, which is influenced by limited funding, is whether to proceed with adaptation of the currently implemented model and then make the new changes in that version, or wait a few months until the new and more conceptually sound version of ELM is available and then modify that version. The best representation of the effects of $SO_2$ will be incorporated into the new version. In either case, the data-based information needed for adaptation will be essentially the same as that now being completed.

## DEVELOPMENT AND IMPLEMENTATION OF SIMULATED DIRECT EFFECTS OF ATMOSPHERIC $SO_2$

Currently the NREL Grassland Biome staff is modifying the structure of the primary producer submodel to more accurately represent the biomass dynamics of grassland ecosystems. A current draft of the new structure is shown in Figure 16.1. The most important changes to be incorporated will be (a) the



$CO_2$ Atmospheric
Photosynthetic Pool
Fruit
Mature Leaves
Juvenile Leaves
Annual Stems and Flowers
Perennial Stems
Live Roots
Recent Standing Dead
Old Standing Dead

Figure 16.1. Structure of the new version of the primary production submodel now being implemented by the NREL modeling staff. The significant changes in the structure are the addition of an explicit photosynthetic pool compartment and the splitting of the existing leaf compartment into two compartments, mature and juvenile leaves.

photosynthetic production of a pool of translocatable carbohydrates and (b) the partitioning of this photosynthate among various portions of the plant. The investigators feel that the new treatment of these two processes in plant production will be especially well suited for showing the direct effects on plants of inorganic sulfur taken up as sulfur dioxide. It is hypothesized that a close interrelationship exists between the process of photosynthesis and partitioning of photosynthate. Field experiments for summer of 1976 are being formulated to test these hypotheses.

A new feature of modeling mature leaves separate from juvenile leaves will also be amenable for showing the effects of sulfur taken up as sulfur dioxide, since these two leaf categories are expected to exhibit differential effects. Related to this concept is the phenology of the plant and the varying effects with the age of the plant. The plant phenology model will also be improved before the final coupling to the sulfur cycling mode.

Other effects on primary producers are expected to be shown on the processes of (a) transpiration, as reflected by changes in stomatal resistance, (b) uptake of sulfate from the soil as reflected by changes in the sulfur status of the plant, and (c) plant death, as reflected by chlorosis and necrosis.

If significant differences are shown to exist among major species of the mixed-grass prairie with regard to sulfur dioxide uptake rates and related effects on the above-mentioned processes, the primary producer model will be able to reflect these changes as altered community composition. Modeled functional groups would be selected with regard to similarities of fundamental production processes, and similarity of responses to sulfur dioxide.

## DEVELOPMENT OF SULFUR SUBMODEL TO EXAMINE SULFUR CYCLE

The direct effects of sulfur dioxide pollution on primary production will depend not only on the amount of sulfur dioxide present in the atmosphere, but also on its distribution throughout the plant system, and the closely related soil system. A sulfur cycling model is ideally suited to demonstrate these relationships, and a current draft of its structure is shown in Figure 16.2. The state variables and processes are selected to maintain a level of resolution comparible with ELM, and similarity exists with the existing nitrogen and phosphorus models. Not only will the effects of pollution be shown on primary producers, but also on the entire sulfur cycling system, a capability set in a more entire ecology perspective.

Atmospheric sulfur inputs will be in the form of sulfur dioxide, and the acid-rain sulfate. Rainfall inputs will be simply a function of rainfall, allowing coupling to atmospheric models which facilitate prediction of concentrations in rainfall. Gaseous inputs to plants might be described by the following scheme:

$$V_G = \frac{1}{r_s} + \frac{1}{r_a} + \frac{1}{r_{int.}} + \frac{F}{C(Z)}$$

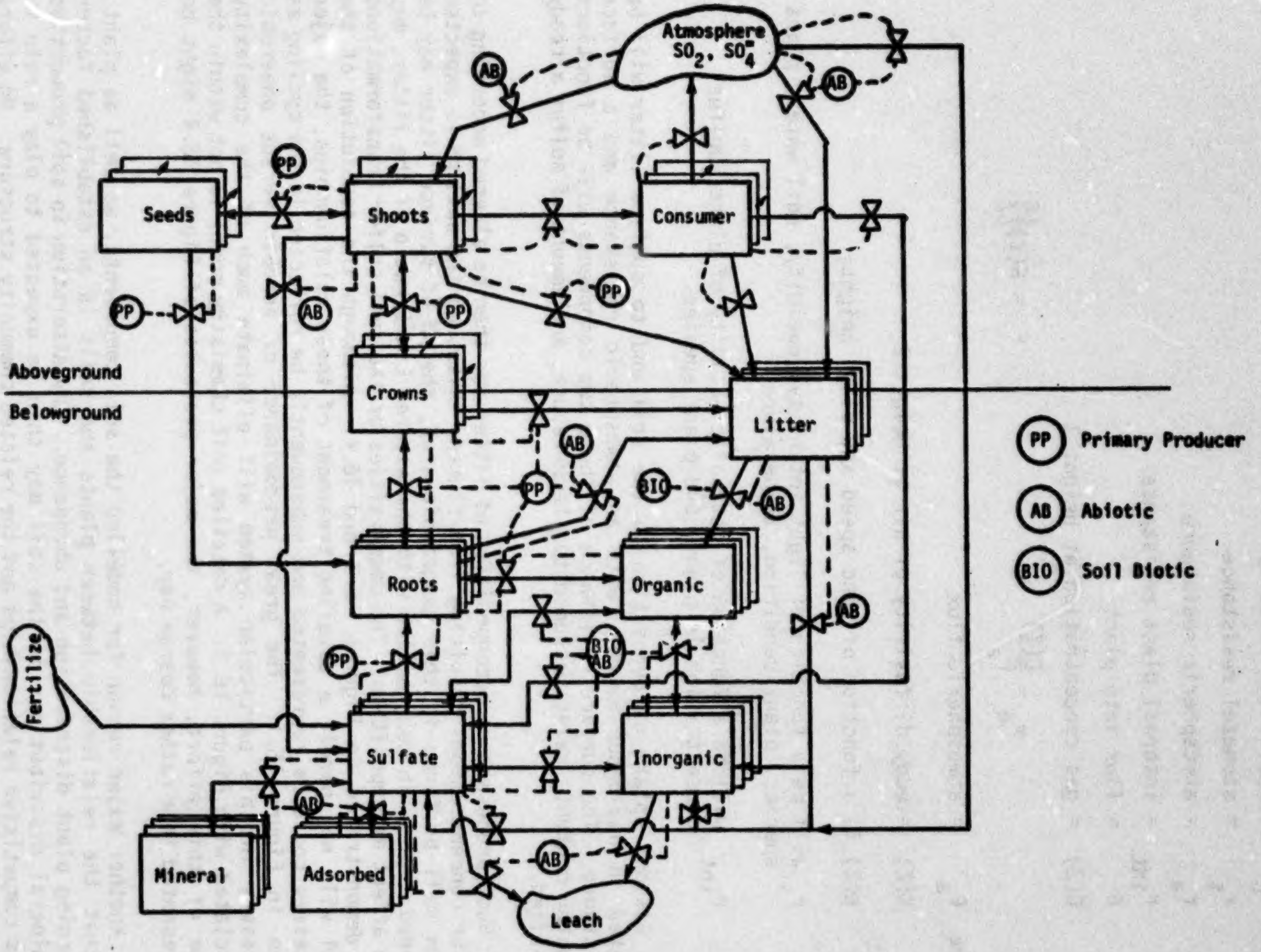

Atmosphere
$SO_2$, $SO_4^=$
AB
AB
Seeds
PP
Shoots
Consumer
PP
AB
PP
PP
Aboveground
Belowground
Crowns
Litter
AB
PP
BIO
AB
Roots
Organic
AB
BIO AB
PP
Fertilize
Sulfate
Inorganic
AB
Mineral
Adsorbed
AB
Leach
PP Primary Producer
AB Abiotic
BIO Soil Biotic

Figure 16.2. Structure of the sulfur submodel for ELM now being developed to represent the dynamics of sulfur cycling in grassland ecosystems.

Where $V_G$ = deposition velocity
$r_s$ = stomatal resistance
$r_a$ = atmospheric resistance
$r_{int.}$ = internal plant resistance
$F$ = flux into plant
$C(Z)$ = gas concentration at height Z

$$r_a = \frac{C(Z)}{F_a} \qquad\qquad F = K(Z)\frac{\delta C}{\delta Z}$$

Where $F_a$ = atmospheric flux

$K(Z)$ = eddy diffusivity of air at height Z

$K(Z)$ is a function of wind speed and plant height;

$r_s$ will be a function of light intensity, humidity, soil water, plant species, plant condition, temperature;

$r_{int.}$ will be a function of metabolic activity of plant, sulfur concentration in plant, and plant species.

Gaseous uptake or deposition to the soil and to surface litter will be treated in analogous manner, with an atmospheric resistance and a surface resistance. The surface resistance of these two components will be functions of water content, relative humidity, temperature, and amount of sulfur already deposited.

Such processes as decomposition of litter may then be altered according to sulfur concentrations resulting from fluxes. Although the soil pH is expected to be well buffered in this geographic area, the pH of surface litter may be altered. This, in conjunction with the altered C:N:S ratio of the litter, may then affect decomposition. The complexities of the soil sulfur transformations are demonstrated in Figures 16.3 and 16.4. Although the resolution of the model will not permit a detailed treatment of these relationships, the major processes could be represented and subsequently be reflected in the cycling as shown in Figure 16.1. The great predominance of aerobic versus anaerobic processes in this particular system will eliminate much of the complexity associated with Figure 16.3. A detailed soil chemistry model is not within the scope of this effort, however, the major processes of Figure 16.4 might be represented in a rather coarse way.

Another major reason for modeling the soil components, as well as plant, is that the relationship between plants and soils is an established factor governing plant distribution and abundance. Any alteration in soil properties or mineral distributions in the soil may then be expected to play a role in plant competitive relationships and the related community structure. No effort is expected to model direct toxic effects of sulfur dioxide upon consumers in this model, only the consumer relationships to the system by way of plant consumption.

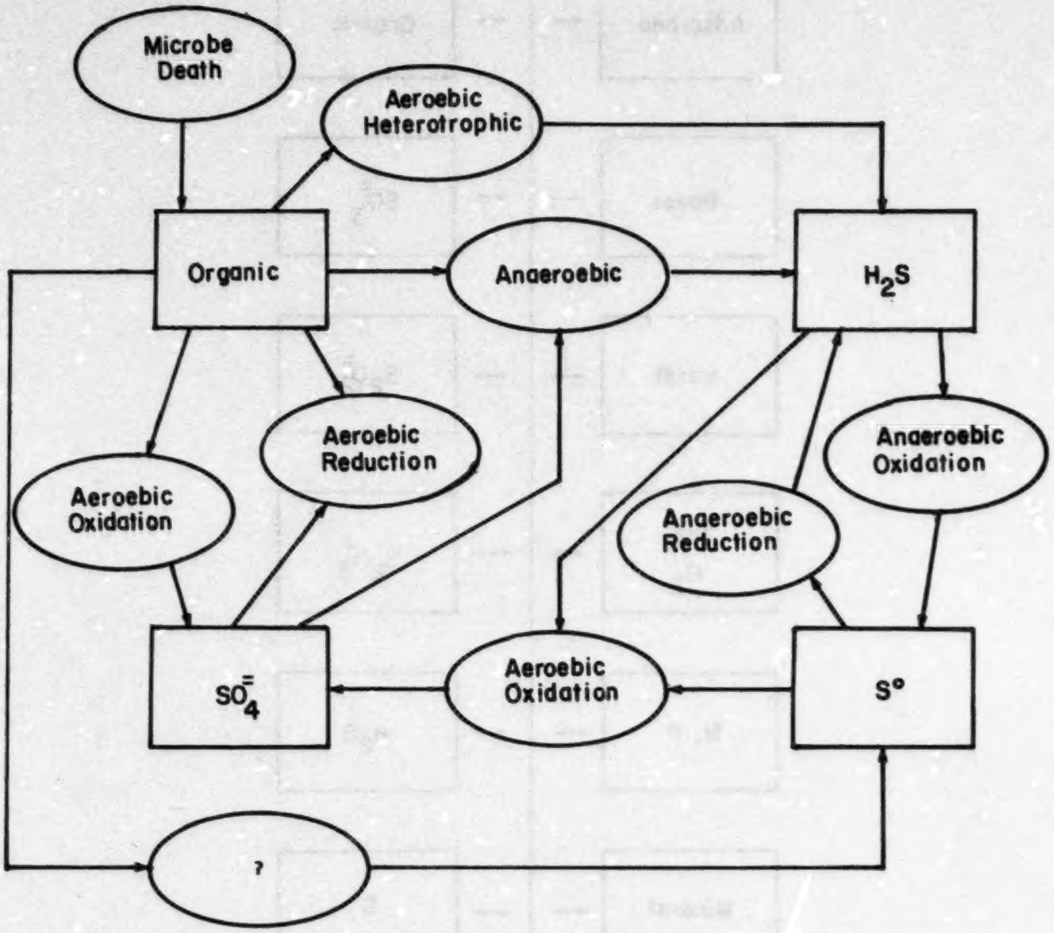

Microbe Death
Aeroebic Heterotrophic
Organic
Anaeroebic
$H_2S$
Aeroebic Reduction
Anaeroebic Oxidation
Aeroebic Oxidation
Anaeroebic Reduction
$SO_4^=$
Aeroebic Oxidation
S°
?

Figure 16.3. Conceptual high resolution of sulfur transformations that potentially occur in soils.

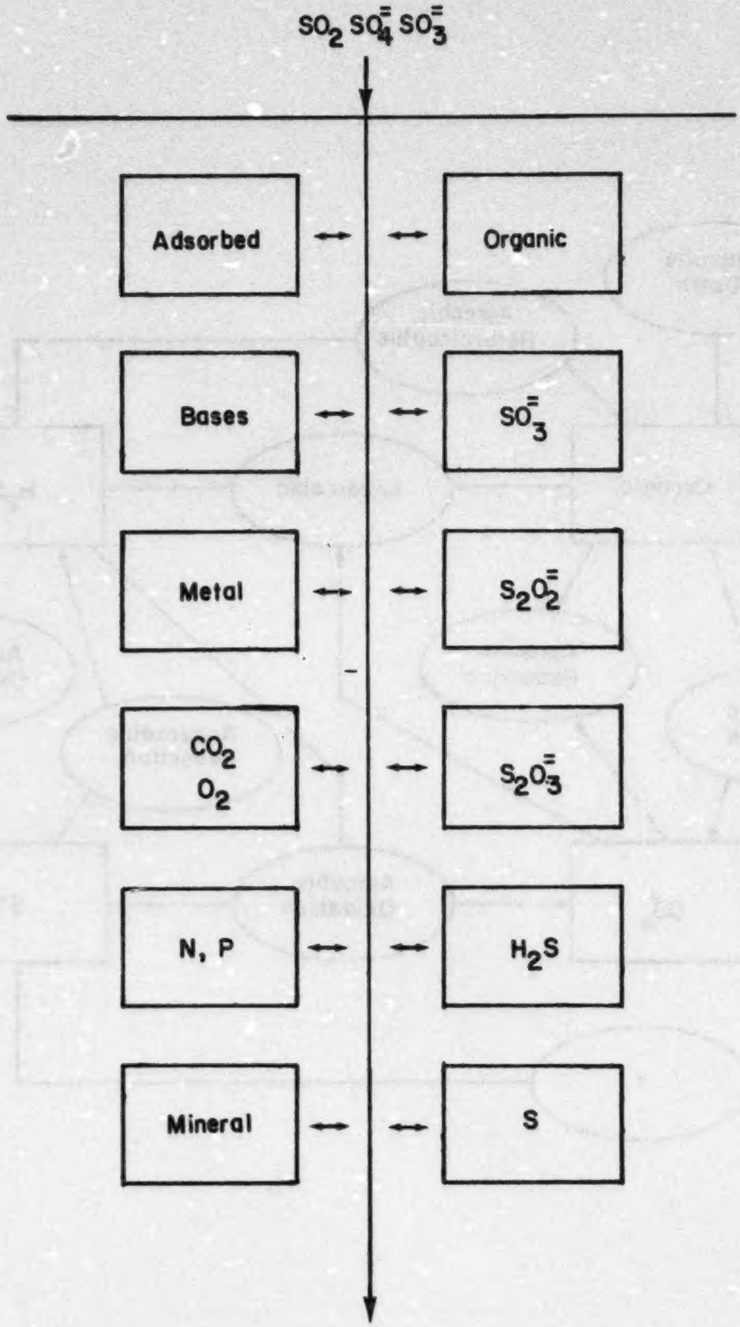

$SO_2$ $SO_4^=$ $SO_3^=$
Adsorbed
Organic
Bases
$SO_3^=$
Metal
$S_2O_2^=$
$CO_2$ $O_2$
$S_2O_3^=$
N, P
$H_2S$
Mineral
S

Figure 16.4. Schematic to represent the complexity of chemical reactions that can occur in soils.